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Prólogo

En el vasto mundo de la biotecnología y la química aplicada, la búsqueda de nuevos compuestos bioactivos con aplicaciones en la salud humana y procesos industriales sigue siendo una prioridad. Este número de nuestra revista se centra en la riqueza de los recursos naturales y su potencial para el desarrollo de tecnologías innovadoras que aprovechen sus beneficios.

Desde los cactus comestibles y sus frutos, fuente de compuestos bioactivos prometedores para la salud, hasta el estudio de los biofloculantes derivados del *Aloe vera*, los artículos que presentamos abordan tanto el impacto de los métodos de extracción como la caracterización fisicoquímica de estas biomoléculas. También exploramos el valor biológico de los residuos de piña, una alternativa sustentable para la obtención de nutrientes esenciales y compuestos funcionales.

Flourensia cernua, una especie vegetal con propiedades biológicas destacadas, protagoniza dos investigaciones que analizan su potencial bioactivo, ya sea mediante un enfoque tradicional o a través de procesos fermentativos innovadores con kombucha. Estos estudios reflejan el creciente interés por aprovechar las propiedades de los recursos naturales mediante bioprocesos eficientes.

Así, este número de la revista invita a la comunidad científica y tecnológica a seguir explorando alternativas sostenibles y eficaces en la obtención de compuestos bioactivos con aplicaciones diversas. La intersección entre naturaleza y ciencia sigue demostrando que en ella se encuentran las claves para el desarrollo de productos y procesos de gran impacto.

Esperamos que estas investigaciones estimulen el diálogo y la generación de nuevas ideas dentro del ámbito de la biotecnología y la química aplicada. ¡Bienvenidos a esta edición dedicada a la innovación en bioprocesos!

Atentamente.

Dra. N. Paola Meléndez Rentería
Editora en Jefe de JBCT

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Edible Cacti and Their Fruits: A Potential Source of Bioactive Compounds for Human Health

Cactus Comestibles y Sus Frutos: Una Potencial Fuente de Compuestos Bioactivos Para la Salud Humana

C. Contreras, S. A.[1], González-Martínez, D. W.[1], Casas-Rodríguez, A. D.[1], Morales-Martínez, T.[1], Rodríguez-Herrera, R.[1], Barrera-Martínez, C.[2], Sepúlveda, L.[1]*

[1]*Departamento de Investigación en Alimentos, Facultad de Ciencias Químicas Universidad Autónoma de Coahuila, Blvd. Venustiano Carranza and J. Cárdenas s/n, Col. Republica Oriente, 25280 Saltillo, México.*
[2]*Centro de Investigación para la Conservación de la Biodiversidad y Ecología de Coahuila, Universidad Autónoma de Coahuila, Miguel Hidalgo 212, Cuatro Ciénegas de Carranza, Zona Centro, 27640 Cuatro Ciénegas de Carranza, México.*

**Corresponding Author:* leonardo_sepulveda@uadec.edu.mx

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Resumen
En esta revisión se examinan los frutos comestibles de los cactus *Stenocereus queretaroensis*, *Cereus repandus*, *Ferocactus pilosus*, *Eulychnia acida*, *Opuntia ficus-indica*, *Hylocereus undatus*, *Matyllucactus geometricans* y *Carnegiea gigantea*, apreciados tanto por su sabor como por sus beneficios para la salud. Estos cactus se distribuyen principalmente en las regiones áridas y semiáridas de América, sobre todo en México y Sudamérica. El consumo de estos frutos ha formado parte tradicionalmente de la dieta de las comunidades locales debido a sus propiedades nutricionales y bioactivas. Entre los compuestos bioactivos presentes en estos frutos destacan las betalaínas por sus propiedades antioxidantes y antiinflamatorias. Las betalaínas han demostrado su eficacia para neutralizar los radicales libres y reducir la inflamación, lo que contribuye a la prevención de enfermedades crónicas. Se discute el potencial de estos cactus para la industria alimentaria y farmacéutica, destacando la importancia de su conservación y uso sostenible para garantizar la sostenibilidad de estas especies de cactus y la preservación de sus hábitats naturales. La sobreexplotación y la expansión agrícola pueden poner en peligro estas plantas, por lo que es importante promover prácticas de cultivo y recolección que respeten el equilibrio ecológico.

Palabras clave: Betalainas, Alimentos funcionales, Nutraceúticos, Farmaceúticos, Metabolitos secundarios.

Abstract
This review examines the edible fruits of the cacti *Stenocereus queretaroensis*, *Cereus repandus*, *Ferocactus pilosus*, *Eulychnia acida*, *Opuntia ficus-indica*, *Hylocereus undatus*, *Matyllucactus geometricans*, and *Carnegiea gigantea*, appreciated both for their flavor and health benefits. These cacti are distributed mainly in the arid and semi-arid regions of the Americas, especially in Mexico and South America. The consumption of these fruits has traditionally been part of the diet of local communities due to their nutritional and bioactive properties. Betalains stand out for their antioxidant and anti-inflammatory properties among the bioactive compounds in these fruits. Betalains have demonstrated their efficacy in neutralizing free radicals and reducing inflammation, which contributes to preventing chronic diseases. The potential of these cacti for the food and pharmaceutical industry is discussed, highlighting the importance of their conservation and sustainable use to ensure the sustainability of these cactus species and the preservation of their natural habitats. Overexploitation and agricultural expansion can endanger these plants, so promoting cultivation and harvesting practices that respect the ecological balance is important.

Keywords: Betalains, Nutritional Properties, Antioxidants, Pharmaceuticals, Secondary Metabolites.

INTRODUCTION

The cacti family (*Cactaceae*) consists of approximately 111 to 118 genera, which include between 1200 to 1500 species. These are perennial trees or shrubs with leaf spines characterized by their succulent stems. They can also be cladodes (as in the Opuntias) and may be radially folded (as in the barrel cactus), as well as having reduced or absent leaves and flowers with numerous tepals and stamens. Their classification is into four subfamilies: *Pereskioideae*, which has persistent and broad vegetative leaves, no glochids, and exarillate seeds; *Opuntioideae*, with cylindrical, deciduous leaves, specialized glochids, and arillate seeds; *Maihuenioideae*, which has persistent leaves; and *Cactoideae*, with absent leaves and glochids, and exarillate seeds (Simpson, 2010; de Araújo et al., 2021; Carpena et al., 2023). Cacti thrive in desert or semi-desert regions as they adapt to thermal stress through the diurnal closure of stomata, which reduces water loss through transpiration, and the MCA (malate-citrate-aspartate) cycle, which allows them to regulate pH and ion balance in the cytoplasm and vacuoles (Bhattacharyya, 2022). Affect antiproliferative effects of the extracts.

Opuntia ficus-indica

Opuntia ficus-indica, commonly known as nopal or Indian fig, is a cactus species of significant economic and ecological importance (Ferreira et al., 2023). Originating from Mexico, this plant has naturalized in various parts of the world, particularly in arid and semi-arid regions (Zamboni et al., 2024). The flowers of the nopal cactus are large and showy, typically yellow, although they can exhibit shades ranging from white to red. The fruits develop, providing food for local wildlife and human consumption (Brahmi et al., 2022). In addition to its culinary uses, *Opuntia ficus-indica* has applications in traditional medicine, where it is attributed to anti-inflammatory and antidiabetic properties (de Albuquerque et al., 2021). Internationally, approximately 150,000 hectares are dedicated to the commercial cultivation of *Opuntia*. In Mexico, the largest producer and consumer, approximately 72,000 hectares are cultivated for fruit production, primarily of the species *Opuntia ficus-indica*, and around 10,500 hectares for nopalitos (young pads) (Issami et al., 2024).

The plant *Opuntia ficus-indica* is drought-resistant, and irrigation is required during the establishment phase to promote good root development. Once established, the plants require less water, but proper irrigation during the growing season can enhance fruit production and forage quality (Barrientos-Sanhueza et al., 2023). Although the nopal can tolerate poor soils, it responds positively to fertilization. Soil analysis is recommended to determine specific nutrient requirements, and the application of organic fertilizers or compost can improve soil structure and provide balanced nutrition (Alam-Eldein et al., 2021).

The harvest of the tunas (fruit) occurs when they reach full maturity, indicated by their color and ease of separation from the cladode. The fruit must be carefully harvested to avoid damage and handled with care to maintain their quality. After harvesting, the fruits should be sorted, packed, and refrigerated as soon as possible to extend their shelf life and preserve their quality until they reach the consumer (Valadez-Moctezuma et al., 2023). Tunas are known for their low caloric content and richness in essential nutrients. They are an excellent source of vitamin C, vital for the immune system and skin health. They also provide B-complex vitamins such as thiamine and riboflavin, which are important for energy metabolism and neuronal health (de Wit & Fouché, 2021). Furthermore, prickly pears are rich in dietary fiber, which aids in digestion and may contribute to constipation prevention. The bioactive compounds in prickly pears, such as betalains and flavonoids, possess antioxidant properties that protect against cellular damage and reduce the risk of chronic diseases, including heart disease (Bouazizi et al., 2020).

Scientific research has explored these benefits, emphasizing the antioxidant activity of prickly pears and their potential as a functional food (Sirotkin, 2022). A study conducted in Mexico and other countries identified that certain varieties of prickly pears exhibit significant antioxidant activity, suggesting their use in creating value-added functional food products (Bouazizi et al., 2020). Another study compared the antioxidant capacity of *Opuntia apurimacensis* and *Opuntia ficus-indica*, finding that both have beneficial compounds, albeit with varying concentrations of vitamin C and total polyphenols (Jorge & Troncoso, 2016).

Hylocereus undatus

The plant *Hylocereus undatus*, belonging to the cactus family, is known for its fruit called pitaya, also known as white dragon fruit. It is found in tropical areas of Africa, South America, and South Asia—countries with tropical climates (Li et al., 2023). The pitaya is an exotic fruit with juicy and sweet flesh. While it is popular for its taste and appearance, it has also been researched for its potential medicinal benefits. Its fruits are consumed fresh or processed into juices, jams, or ice creams (Asghar et al., 2024). The use of *Hylocereus undatus* for medicinal purposes is documented in traditional medicine systems. Several compounds in *Hylocereus undatus* contribute to its biological activities. In southern China, the flowers of *H. undatus* have been used as food and popular medicine to treat various conditions (Li et al., 2023).

The pitaya is highly nutritious, with a high water content, natural sugars, minerals, and antioxidants, while being low in calories. It also contains seed oil, betacyanins, triterpenoids, and steroids, which have laxative, hypocholesterolemic, anti-inflammatory, anticancer, and antidiabetic effects. Due to its functional and medicinal attributes, pitaya has excellent potential for use in the food, pharmaceutical, and cosmetic industries. However, further research is needed to obtain consistent and reliable data, allowing unrestricted exploration of its use (Michelle et al., 2017).

In a recent study, three new glucosides were isolated from the flowers of this plant, along with other known compounds. These glucosides exhibited antioxidant, anti-inflammatory, and antitumor properties (Havas et al., 2023). These antioxidants help protect cells from oxidative stress caused by free radicals, reducing the risk of chronic diseases such as cardiovascular diseases, diabetes, and cancer (Nur et al., 2023). *H. undatus* is also rich in bioactive compounds. It has been reported that this plant contains bioactive compounds such as vitamins, saponins, and condensed tannins. Additionally, it presents molecules like betacyanin, *p*-coumaric acid, vanillic acid, and gallic acid. Dry pitaya peel powder has been used for rumen fermentation, enhancing gas production and reducing methane concentration. Therefore, pitaya has the potential to be utilized as a feed additive to mitigate methane emissions from ruminants (Matra et al., 2019; Nishikito et al., 2023). Furthermore, *Hylocereus undatus* has demonstrated antimicrobial properties, showing activity against various types of bacteria and fungi, which makes it potentially valuable for infection treatment (Clemente Jr et al., 2021).

In another study, the presence of betacyanin in *H. undatus* was investigated. Betacyanin is uniformly distributed in the shell cells and remains intact in the vacuole. The estimated concentration in the shell is 95 ± 0.873 mg/g fresh weight. In addition, the pigment extract is stable with ascorbic acid and citric acid. Betacyanin inhibits xanthine oxidase with an IC50 of 9 mM. It could be a natural inhibitor to prevent uric acid-related diseases (Dey et al., 2022). In the food industry, *H. undatus* has been used to develop edible films. The authors utilized pitaya peels, mixed them with distilled water in a 1:2 ratio, and subjected them to ultrasonication for 30 minutes to obtain the mucilage. Glycerol and pectin were added to enhance its properties without compromising visual appeal. Furthermore, these films are biodegradable (López-Díaz et al., 2023).

Myrtillucactus geometrizans

Myrtillocactus geometrizans, also known as Garambullo, blue myrtle cactus, or Mesoamerican blue cactus, is a succulent plant belonging to the cactus family. The fruits are small and round and dark red when ripe (Sanjuan-Trejo et al., 2021). "Garambullo" is native to arid and semi-arid regions of Mexico and Central America. It is found mainly in desert areas, mountain slopes, and ravines. In addition, it can withstand high temperatures and can survive in drought conditions. It can be found at altitudes ranging from sea level to 2,500 meters (Sandoval-Gallegos et al., 2022).

Garambullo fruits are consumed fresh and are used in the preparation of jellies, jams, liqueurs, ice creams, and traditional dishes. They stand out for their sweet and sour flavor and high content of betalains, compounds with nutritional value. The fruits are rich in vitamin C, fiber, and antioxidants, which make them beneficial to health, including the treatment of diabetes and cancer prevention. Dehydration is a technique used to prolong the fruit's postharvest life, allowing garambullo raisins to be produced (Herrera-Hernández et al., 2011; Montiel-Sánchez et al., 2021a).

The cactus *M. geometrizans* has a long history of traditional and medicinal uses. In traditional medicine, it has been used for various purposes, including treating various ailments such as diabetes, diarrhea, inflammation, fever, fungal infections, bacterial infections, and liver protection. Some compounds identified in *M. geometrizans* are polysaccharides, flavonoids, phenolic acids, and alkaloids (Herrera-Hernández et al., 2011; da Silveira Agostini-Costa, 2020). These compounds contribute to the pharmacological activities of the plant, which have been extensively studied. Studies have shown garambullo exhibits antidiabetic activity by improving insulin sensitivity and lowering blood glucose levels. In addition, the plant has demonstrated antioxidant properties, which help neutralize harmful free radicals and protect against oxidative stress (López-Palestina et al., 2018). The mechanisms of action underlying these bioactivities are still under investigation. However, some studies suggest that Garambullo bioactive compounds may act by modulating various molecular targets and signaling pathways. For example, polysaccharides from *M. geometrizans* have been found to stimulate insulin secretion and enhance glucose uptake into cells (Reynoso-Camacho et al., 2015).

In addition, flavonoids present in *M. geometrizans* may exert their antioxidant and anti-inflammatory effects by inhibiting reactive oxygen species and proinflammatory cytokines. The phenolic acids in garambullo have also been shown to have anti-inflammatory and antimicrobial properties, possibly through inhibition of the activity of enzymes involved in inflammation and disruption of microorganism cell membranes. *M. geometrizans* has a wide range of traditional and medicinal uses due to its bioactive compounds. These compounds contribute to the pharmacological activities of the plant, which have been extensively studied. (Céspedes et al., 2005; Salazar et al., 2011; Domenico Prisa, 2021)

Carnegiea gigantea

Carnegiea gigantea, commonly known as saguaro, is a large columnar cactus that can reach impressive heights, emblematic of desert regions of North America, and is native to the Sonoran Desert in Arizona, the Mexican state of Sonora, and some areas of California. The saguaro is known for its extremely slow growth, reaching 1 meter in about 25 years. Its longevity can be 150 to 200 years, and some specimens reach 16 to 18 meters in height. This cactus can withstand significant temperature differences, from -9 °C to over 50 °C (Renzi et al., 2019). Saguaro has been widely used by the indigenous peoples of Sonora, such as the Papago, who consume its fruits, flowers, and seeds, which are rich in sugars and are a fundamental part of their diet. *C. gigantea* is known for its traditional uses by indigenous peoples, who have used various parts of the cactus for medicinal purposes (Jiménez-Sierra et al., 2023). For example, the fruit of *C. gigantea* has been used for its high vitamin C content and as a remedy for indigestion and gastrointestinal problems. The flowers and stems have also prepared teas or infusions for their diuretic and laxative properties. In addition, the cactus is considered to have anti-inflammatory and antioxidant effects due to its phenolic compounds (Zheng et al., 2021).

In addition to its traditional uses, *C. gigantea* has also been studied for its potential pharmacological properties. Biochemical analysis has revealed the presence of various bioactive compounds, such as phenolic compounds, flavonoids, and polysaccharides. These compounds are believed to contribute to the pharmacological activities of this cactus, which have been demonstrated in several studies. For example, studies have shown that *C. gigantea* extracts exhibit antioxidant activity, which may help protect against oxidative stress and reduce body inflammation. In addition, the extracts have been found to have antimicrobial properties, inhibiting the growth of certain bacteria and fungi (Tamayo Ordoñez et al., 2023).

Eulychnia acida

The cactus *Eulychnia acida*, known locally as 'copao,' is a columnar cactus that varies in shape, generally arboreal, 1.5-4 meters high, with a defined trunk 1 meter long and then more or less branched, forming a rounded crown, but sometimes shrubby, very branched, without trunk, forming a low mass of 1 meter high or less, with branches often prostrate or ascending. It belongs to the Cactaceae family and is native to arid and semi-arid regions. A perennial plant thrives in dry, harsh conditions, making it well-adapted to survive in arid climates (Cares et al., 2018). This cactus is endemic to western Chile, which can be found in Coquimbo and up the middle of the Atacama region. It grows from sea level to 1,300 meters above sea level. It prefers rocky slopes where fog and mist condense on the plant's branches and nearby rocks and drip down to the roots, but also on gentler slopes and even plains, only where there is some rain. Even in these locations, plants rarely receive more than 50 mm of rain annually (Salvatierra, 2020).

The 'copao' cactus is mainly consumed for its fruit. The fruit is eaten fresh and is known for its tart and refreshing flavor. In the regions where it grows, it is consumed locally, and juice is extracted from the fruit, which can be drunk directly or used as an ingredient in mixed drinks. In addition, jams and preserves are made with the fruits. Traditionally, local medicine has used the fruit to treat various ailments, although caution should be exercised. (Masson S et al., 2011). Scientific research on the benefits of copao has highlighted that the fruit of the *Eulychnia acida* cactus is a good natural source of vitamin C and has a high sodium and potassium content, which helps regulate water balance through a rehydrating effect. It also contains minerals lost through sweating, provides soluble dietary fiber, is low in calories, and contains antioxidant compounds. These properties give copao great potential as a superfood and raw material to produce higher value-added products (Jiménez-Aspee et al., 2015).

These benefits make copao an interesting option for food and for exploring its medicinal properties. In addition, the exploitation of copay is still low, and its harvesting is quite artisanal, so efforts are being made to better understand its commercial potential and improve its agronomic management (Venegas et al., 2016).

Cereus repandus

Cereus repandus, commonly known as the Peruvian cactus or Peruvian apple, is a columnar cactus species found in various regions of South America (Singh Yadav et al., 2024). Its presence is notable in Brazil, Uruguay, and Argentina, where it adapts to various environments, from arid areas to dry forests (De Faria-Tavares et al., 2013).

Cultivation is relatively simple, as this cactus prefers well-drained soil and direct sun exposure, although it tolerates partial shade. It is drought-resistant thanks to its ability to store water in its fleshy tissues, allowing it to survive prolonged periods without irrigation. However, for optimal growth, moderate watering during the growing season and a significant reduction during the winter is recommended (Yang & Pritchard, 2022). The flowers of *Cereus repandus* appear mainly on warm summer nights, opening at dusk and closing at dawn. These nocturnal flowers are large, up to 15 centimeters in diameter, and feature pure white petals that contrast with the brownish-green center (Becker et al., 2023).

Pitaya is a nutrient-rich, low-calorie food. It contains vitamins such as vitamin C, which is essential for the immune system, and significant amounts of vitamin B, which aids in cellular metabolism. In addition, it is a good source of minerals such as calcium and iron. The presence of antioxidants such as betalains and carotenoids helps neutralize free radicals in the body, which can reduce the risk of chronic diseases. (de Alcântara Oliveira et al., 2020).

Stenocereus queratorensis

Stenocereus queretaroensis, also known as pitayo or yagüarey, is a cactus found in arid areas of central and northern Mexico. It has an arboreal appearance and is robust. During spring, it flowers and produces fruits that ripen in April and May. The fruits of *S. queretaroensis* are globose or ovoid, with spiny areoles. They can range in color from carmine to red to white. They contain numerous black seeds, and their weight varies between 85 and 400 grams (García-Cruz et al., 2022). Pitayos play an important role in Mexican culture and tradition. They have been consumed since pre-Hispanic times and have been used as medicine. Some people obtained pitayo flour and even used the seeds. It has been demonstrated that these uses are due to the high content of phenolic compounds and betalains in the fruits, which causes a high antioxidant potential, being also a source of natural pigments with potential application in the food industry (Delia et al., 2019).

The fruit of *Stenocereus queretaroensis* is rich in betalains, water-soluble compounds responsible for the fruit's vibrant colors. Studies suggest that these betalains have health benefits, such as cardiovascular and anti-inflammatory properties. In addition, cells high in betalains show antimicrobial and antifungal activities (Ceja-López et al., 2022; Gonzalez-Campos et al., 2022). The study used phytochemicals extracted from this fruit peel to synthesize silver nanoparticles. These nanoparticles demonstrated antimicrobial activity against gram-negative bacteria (*E. coli*, *S. enterica*, and *P. aeruginosa*) and gram-positive bacteria (*S. aureus* and *MRSA*). The authors suggest that these nanoparticles could be helpful in the food industry, either as coatings for food packaging or as disinfectants on different surfaces. (Padilla-Camberos et al., 2021).

Ferocactus pilosus

Red biznaga, or biznaga cabuchera (*Ferocactus pilosus*), is a cactus native to America. It grows solitary or in large groups and is cylindrical, with diameters up to 50 cm. The cactus is endemic to the northern part of the Sierra Madre Oriental and the Chihuahuan Desert (Mascot-Gómez et al., 2021). *F. pilosus* has flowers approximately 4 cm long; they do not open completely because thorns surround them, and these flowers can be red or yellow. The flowers give way to an ovoid fruit covered with yellow bracts. The flower buds of this cactus are known as cabuches and are famous for their nutritional properties and culinary potential. Cabuches are harvested raw in the spring, from March to May, are cooked in different ways, and are characterized by their texture similar to that of asparagus and their slightly acidic and herbaceous flavor (Díaz-Reyes et al., 2020). Cabuches are rich in fiber, vitamin C, and minerals. They are traditionally attributed to a diuretic effect, which may help eliminate toxins from the body. These fruits also contain antioxidants that may help protect against cell damage. Some studies also suggest that compounds present in cabuches may have anti-inflammatory properties (Salas Hernández, 2023b). The cabuchera biznaga is a protected species, so it is important to harvest cabuches responsibly and sustainably. For this reason, these fruits are not currently exploited, but research is being carried out to propagate these cacti in vitro (Salas Hernández, 2023a). Figure 1 shows a summary of the main cacti.

Compounds in common within the genus Cactaceae

The compounds in the family *Cactaceae* are varied and have different biological activities. The biological activities of the compounds in the Cactaceae family are of interest to scientific studies. Cacti contains various bioactive compounds, most notably phenolic acids and flavonoids (Agostini-Costa, 2022). Figure 2 shows some chemical structures of these compounds; among the different compounds found in cacti, a very particular family stands out: the Betalains (Sadowska-Bartosz & Bartosz, 2021).

Betalains are pigments present in a tiny group of botanical families. These pigments are responsible for the colors of flowers and fruits, especially in plants such as cacti. Betalains accumulate in the vacuoles of plant cells and contain nitrogen. They also possess antioxidant properties and act as a barrier against UV rays. Betalamic acid is a key component in their structure (Figure 3-a) (Dias et al., 2016). Betalains are subdivided into betacyanins and betaxanthins (Figure 3-b,c). The betacyanins (Figure 3-b) are red to violet-colored compounds with an absorption maximum of 540 nm (Madadi et al., 2020). Betacyanins are formed from betalamic acid and cyclo-DOPA, to which sugar or hydrogen molecules can be attached as substituents. Betaxanthins (Figure 3-c) are yellow to orange compounds with an absorption peak in the visible spectrum at 480 nm. Betaxanthins result from the condensation of amines and betalamic acid, and an amine group and hydrogen usually join this nitrogen as substituents (Valencia et al., 2017).

These compounds, like polyphenols, are more stable due to the following factors: low water activity, pH between 3 and 7, low temperatures, darkness, and absence of oxygen. Betalains undergo different reactions that are degradation pathways, which can be dehydrogenations or decarboxylations (Polturak & Aharoni, 2018).

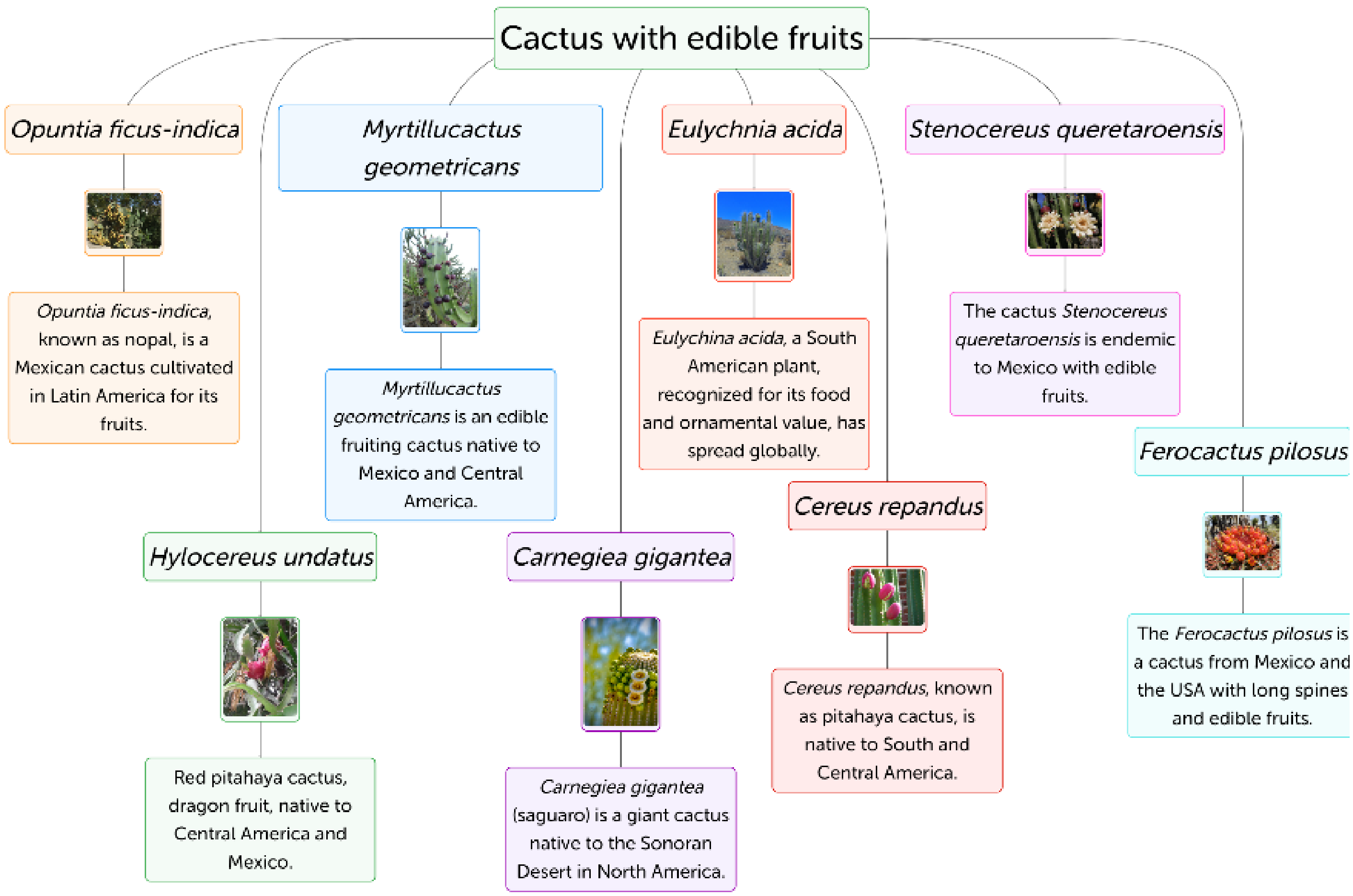

Figure 1. Main cacti with edible fruit are present in Mexico.

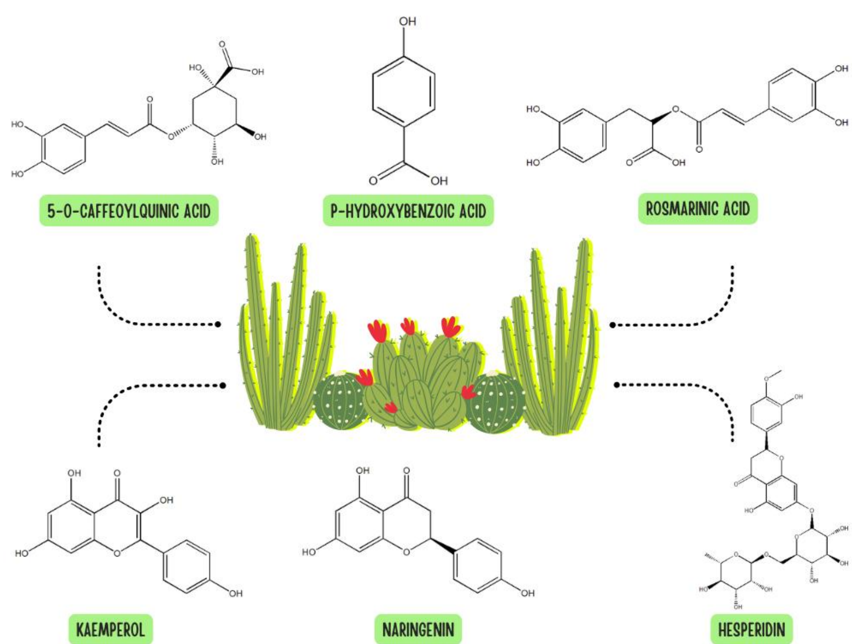

Figure 2. Main phenolic acids and flavonoids present in cacti.

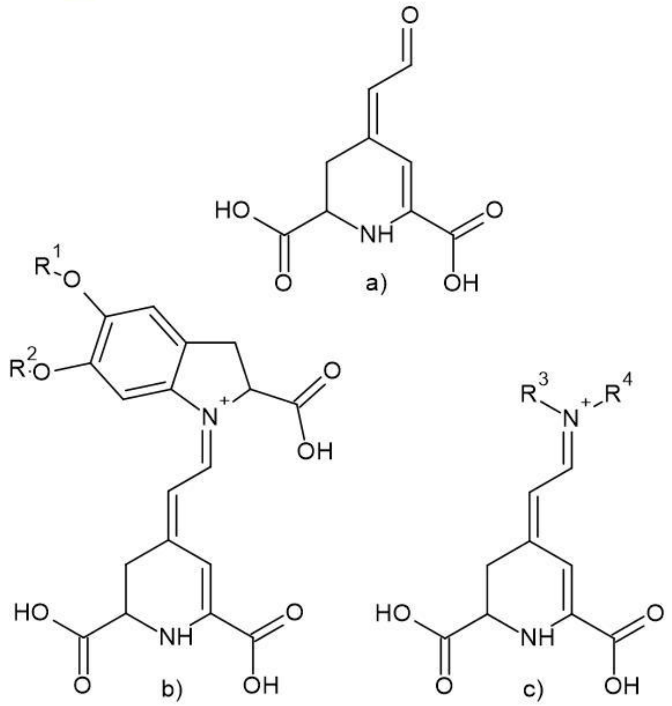

Figure 3. Chemical structures of betalamic acid (a), betacyanin (b), and betaxanthins (c) R[1] and R[2] are sugars or hydrogen; R[3] is an amine group or amino acid; R4 is mostly hydrogen

Health benefits

Betalains, in addition to pigmenting, have different properties, such as antioxidant and antimicrobial. One of the derivatives of betalains commonly used in the cosmetic industry is betacyanin (Abedi-Firoozjah et al., 2023). These compounds have a condition of structural degradation with time, where the color change becomes noticeable with temperature change, which would be optimal for use as time-temperature indicators in food. As well as for the development of intelligent packaging, as reported in works, since it is indicated that they have biological properties such as anti-radical, anticarcinogenic, and anti-lipidemic (Carreón-Hidalgo et al., 2022). Betalains have different biological properties, many of which contribute to preventing degenerative diseases. Betalains have the property of being useful in the prevention of some types of cancer, cardiovascular and gastrointestinal diseases, as well as diabetes and obesity. The advantage of betalains is that, compared to anthocyanins, they are less susceptible to degradation, which is why they also have applications in the food industry. Like most phenolic compounds, Betalains have a high antioxidant capacity, as they can capture reactive oxygen species and protect against lipid peroxidation catalysts (Rodrigues Vieira et al., 2024).

Betalains have been investigated as they demonstrated anti-inflammatory activity, and the ability to inhibit the expression of intercellular adhesion molecule (ICAM-1) in TNF-α-stimulated human endothelial cells has been tested. The activity of betatine was higher, even at micromolar concentrations (Gentile et al., 2004). Studies have shown that beet juice rich in betalains has an effect on the formation and release of interferon-γ, which results in the reduction of reactive oxygen species (ROS) formation, preventing conditions related to oxidative stress, such as cancer, atherosclerosis, Alzheimer's and Parkinson's disease (Montiel-Sánchez et al., 2021b). On the other hand, the antioxidant capacity of most betalains and betalamic acid is superior to that of common flavonoids, ascorbic acid, and tocopherols, which is why it is attractive to use extracts of these as artificial colorants (Bastos & Schliemann, 2021).

PERSPECTIVES

Research should begin to focus on the conservation of these species and their biodiversity since some species can be considered endangered, and this can be achieved through genetic improvement to make them resistant to pests and diseases and increase their nutritional value. Sustainable production must also be a priority, as well as improving cultivation techniques that reduce the use of water and resources and the use in arid and semi-arid zones. Finally, biotechnology is the key to making the best use of these cacti; bioplastics and other derived products can be produced, and food applications in the processing of fruits can be used to produce new products.

CONCLUSIONS

Cacti are a valuable source of bioactive compounds, especially betalains. Unlike other pigments such as anthocyanins and carotenoids, these compounds show remarkable physicochemical stability in industrial processes, being a promising alternative for pharmaceutical and cosmetic applications. The tendency to use natural pigments instead of synthetic ones is increasing, which benefits human health and the environment. However, there is still a wide field of research that is needed to develop more environmentally friendly production methods, reduce the energy expenditure in obtaining these compounds, and explore applications in medicine by generating pharmaceuticals and in the food industry with possible food supplements. Research on cacti with edible fruits and polyphenolic compounds continues to be a promising field. The search for sustainable cultivation methods for these cacti, such as in vitro propagation of these cacti and the application of these compounds in medicine and the food industry, are exciting challenges that deserve further scientific research. Innovative processes such as fermentation and supercritical fluid extraction remain to be investigated in this field.

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Potential of pineapple waste: Bioactive compounds and their biological significance in human health

Potencial de los residuos de piña: Compuestos bioactivos y su importancia biológica en la salud humana

Casas-Rodríguez, A.D.[1], González-Martínez, D.W.[1], C. Contreras, S.A.[1], Ascacio-Valdés, J.A.[1], Dávila-Medina, M.D.[1], Chávez-González, M.L.[1], Sepúlveda-Torre, L.[1*]

[1]*Departamento de Investigación en Alimentos, Facultad de Ciencias Químicas Universidad Autónoma de Coahuila, Blvd. Venustiano Carranza and J. Cárdenas s/n, Col. Republica Oriente, 25280 Saltillo, México.*

**Autor para correspondencia: leonardo_sepulveda@uadec.edu.mx*

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Resumen

El documento revisa el potencial de los residuos de piña como fuente de compuestos bioactivos y su importancia para la salud humana. Se resalta que la industria alimentaria genera grandes cantidades de residuos de piña, como cáscara, corazón y corona, los cuales contienen carbohidratos, proteínas, minerales, vitaminas y enzimas con efectos beneficiosos. Los compuestos bioactivos más relevantes identificados en los residuos incluyen polifenoles, taninos y bromelina. Estos presentan propiedades antioxidantes, antimicrobianas, antiinflamatorias, anticancerígenas y antidepresivas. La investigación destaca que estos compuestos pueden ayudar en la prevención de enfermedades crónicas y tienen aplicaciones en las industrias farmacéutica, alimentaria y cosmética. En términos de extracción, se han desarrollado diversas técnicas, desde métodos convencionales como maceración y Soxhlet hasta tecnologías emergentes como ultrasonidos y microondas. Se enfatiza la necesidad de optimizar estos procesos para una mejor obtención y aprovechamiento de los compuestos activos. Los residuos de piña han demostrado ser efectivos en la reducción del estrés oxidativo, la inhibición del crecimiento de bacterias y la modulación de respuestas inflamatorias. La bromelina, en particular, ha sido estudiada por sus efectos contra infecciones y enfermedades neurodegenerativas. Además, se ha encontrado que los extractos de piña pueden tener efectos antidepresivos al influir en los niveles de serotonina. En conclusión, la valorización de los residuos de piña representa una oportunidad para el desarrollo de productos funcionales y terapias naturales.

Palabras clave: Subproductos, polifenoles, antioxidante, antimicrobiano, antiinflamatorio.

Abstract

This document reviews the potential of pineapple waste as a source of bioactive compounds and its importance for human health. It highlights that the food industry generates large quantities of pineapple waste, such as peel, core, and crown, which contain carbohydrates, proteins, minerals, vitamins, and enzymes with beneficial effects. The most relevant bioactive compounds identified in the waste include polyphenols, tannins, and bromelain. These have antioxidants, antimicrobial, anti-inflammatory, anticancer, and antidepressant properties. The research highlights that these compounds can aid in the prevention of chronic diseases and have applications in the pharmaceutical, food, and cosmetic industries. In terms of extraction, various techniques have been developed, from conventional methods such as maceration and Soxhlet extraction to emerging technologies such as ultrasound and microwave extraction. The need to optimize these processes for better extraction and utilization of active compounds is emphasized. Pineapple waste has been shown to be effective in reducing oxidative stress, inhibiting bacterial growth, and modulating inflammatory responses. Bromelain has been studied for its effects against infections and neurodegenerative diseases. Furthermore, pineapple extracts have been found to have antidepressant effects by influencing serotonin levels. In conclusion, the valorization of pineapple waste represents an opportunity for the development of functional products and natural therapies.

Keywords: Subproducts, polyphenols, antioxidants, antimicrobial, anti-inflammatory.

INTRODUCTION

The pineapple (*Ananas comosus* L.) belongs to the *Bromeliaceae* family, which is made up of 46 genera and 2,000 species (Kavuthodi and Sebastian, 2018). It is one of the most produced fruits in the world, cultivated mainly in Indonesia (3,156, 576 ton in 2023), Philippines (2,944,260 ton in 2023), Costa Rica (2,937,807 ton in 2023), Brazil (2,387,tons per years), China Continental (2,093,596 ton in 2023), India (1,828,000 ton in 2023), Nigeria (1,615,622 ton in 2023), Nigeria (1,615,622 ton in 2023), Mexico (1,272,559 ton in 2023), Thailand (1,258,028 ton in 2023) and Colombia (853,169 ton in 2023) (Aili Hamzah et al., 2021; FAO. FAOSTAT).

However, in recent years the food industry has been growing and with it the demand for food, which has generated greater processing of fruits and vegetables; and the pineapple fruit is no exception since around 25 to 50% of waste is generated, corresponding to the peel, heart, crown and leaves (Banerjee et al., 2018; Kocakaplan et al., 2024; Sánchez-Hernández et al., 2015). This type waste generate great interest in recent years due to contain carbohydrates, proteins, cellulose, minerals, vitamins, enzymes and bioactive compounds that benefits to human health (Mohd Ali et al., 2020). To obtain bioactive compounds have been studied different methods, such as conventional ones (maceration or Soxhlet) (Dewi and Simamora, 2023; Muñoz Acevedo et al., 2021), technologies emerging (ultrasound or microwave) (Vargas-Serna et al., 2022; Zampar et al., 2022). As well as biotechnologies (fermentation in solid or liquid state) (Polania-Rivera et al., 2023).In accordance with the above, the objective of the present work was to analyze the bioactive compounds present in pineapple residues and their main biological activities, highlighting their application in the food, pharmaceutical and cosmetic industries, as well as their contribution to human health.

Pineapple (*Ananas comosus* L.)

The pineapple (*Ananas comosus* L.) is a plant belonging to the Bromaliaceas family, which can measure between 1 or 2 m in height. The fruit is made up of the stem, leaves, peduncle, fruit, crown and roots (León-Yáñez et al., 2019; Uriza-Ávila et al., 2018). This fruit is considered rich in flavor and aroma, as well as high nutritional value, depending on the variety of pineapple its characteristics vary (Vieira et al., 2022). For the pineapple to continue to have its characteristic flavors and smells, the geographical area, temperature, humidity, soil properties, as well as daily exposure to the sun, constant watering and an optimal pH must be taken into account (Lobo and Yahia, 2017; Uriza-Ávila et al., 2018).

Composition of pineapple waste and its benefits

Pineapple contains an important nutritional content, which gives the fruit its sweet and juicy properties, in addition to multiple benefits for human health; This can be attributed to the variety and the maturity of the fruit, as well as the climatic conditions and its proper management for harvest (Ajayi et al., 2022; Mohd Ali et al., 2020). Among the main components of pineapple are water (86g/100g), carbohydrates (13.12g/100g), proteins (0.54g/100g), lipids (0.12g/g), fibers (1.54g/100g), minerals (potassium 109mg/100g; magnesium 12mg/100g), and vitamin C (47.8mg/100g) (Uriza-Ávila et al., 2018).

It has been proven that pineapple waste (center, crown and peel) is also rich in carbohydrates, minerals, vitamins and bioactive compounds. Among the benefits of this fruit is that it has anti-cancer properties, is effective as a diuretic, eliminates intestinal worms, prevents ulcers, helps reduce lipids (Banerjee et al., 2018), cardiac oxidative stress, helps constipation and gastrointestinal function, in addition to being antiarthritic, lipid-lowering, antidiabetic (Uriza-Ávila et al., 2018), also having certain benefits in regulating emotional stability (Nurrahma et al., 2024), anti-malarial and anti-inflammatory (Ajayi et al., 2022; Kargutkar & Brijesh, 2018) (Figure1). It has been described that bromelain can treat bacterial infections, bronchitis, pneumonia, sinusitis, parasitic gastrointestinal infection (Mohd Ali et al., 2020).

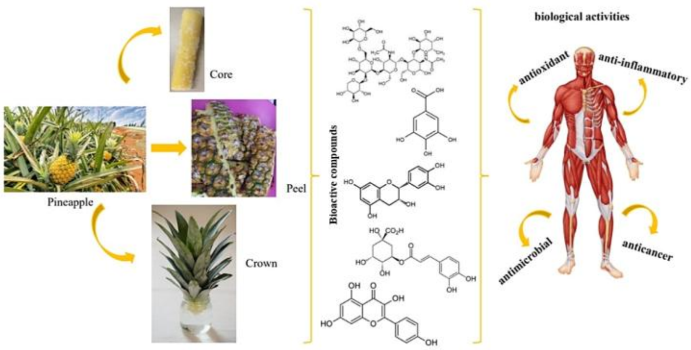


Figure 1. Graph of pineapple bioactive compounds and their biological activities.

Pineapple peel

Within the processing of pineapple, around 25 to 50% of waste is generated and among them is pineapple peel (Sánchez-Hernández et al., 2015). It has been shown that the peel has a high nutritional value, including sugars such as sucrose, glucose and fructose, organic acids, minerals, cellulose, bioactive compounds, among others (Lourenço et al., 2021).Various vitamins such as C, A, and E have been found, as well as carotenoids, oils, fatty acids, hemicellulose (Rico et al., 2020). Although bromelain is the main enzyme in pineapple waste, cellulase, xylanase and pectinase have also been found (Meena et al., 2022a).

Pineapple core

The heart of the pineapple represents around 15% of the pineapple fruit, according to what was studied by (Kodagoda & Marapana, 2017).The proximal analysis of the heart of the pineapple is composed of moisture content (84.90 ± 0.23%), crude protein (3.67 ± 0.05 g/100 g), fat (2.35 ± 0.15 g/100g), crude fiber (9.14 ± 013 g/100g), ash (1.70 ± 0.15 g/100g), carbohydrates (83.03 ± 0.32 g/100g) and minerals such as potassium (247.70 ± 32.80 mg/100g), calcium (8.73 ± 0.34 mg/100g), iron (1.61 ± 0.23 mg /100g) and manganese (1.63 ± 0.54). They also contain various enzymes, such as bromelain, β-glucosidase, cellulase, among others (Banerjee et al., 2018; Paz-Arteaga et al., 2023b). It has been determined that the core of the pineapple has a higher content of glucose and fructose, as well as vitamin C (Polanía et al., 2023). The core of the pineapple is one of the most important wastes since bromelain and fiber are obtained, since it has various applications (Fissore et al., 2023).

Pineapple crown

The crown represents around 2.7 to 5.9% of the fruit (Polanía et al., 2023). The proximal composition of pineapple is composed of protein (0.7%), ash (7.37%), crude fat (3.5%), rough fiber (62.5%) (Baidhe et al., 2021). A composition of carbon (39.50%), hydrogen (5.51%), nitrogen (13.82%) and sulfur (0.46%) has been found, as well as various enzymes such as bromelain and cellulase (Yazid and Roslan, 2020). The pineapple crown is one of the wastes that contains the most fiber, which is why within the paper industry it is a promising waste. The proximal composition of pineapple is composed of protein (0.7%), ash (7.37%), crude fat (3.5%), rough fiber (62.5%) (Baidhe et al., 2021). Table 1 shows the physicochemical composition of different pineapple waste.

Bioactive compounds

It is considered a “bioactive component or compound” of a food, that which brings a health benefit further beyond the considered rated, such as improvement in biological functions or reduction in the risk of suffering from a disease (Polanía et al., 2023). In recent years there has been great interest in fruits and vegetables because they contain this type compounds beneficial for health, likewise, it has been shown that their waste contain in a percentage of bioactive compounds, which is why they have been of interest for study since it has been proven that they can be extracted biomolecules of great value (Kainat et al., 2022; Patra et al., 2022).

Polyphenols

Bioactive compounds known as polyphenols are secondary metabolites that are found mainly in the covering layer of plants and are characteristic for their structures, since they can contain one or more aromatic rings that are linked to hydroxyl groups (Montenegro-Landívar et al., 2021; Nordin et al., 2023). Polyphenols are divided into 5 main groups which are phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), stilbenes, lignans, flavonoids (flavonoids, isoflavones,

Table 1. Physicochemical composition of pineapple waste.

Pineapple waste	Moisture (%)	Total Sugar (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	Reference
Peels	88.40	82.27	8.86	1.89	1.01	1.81	Kumaresan et al., 2025
Peel	81.12	35.57	10.73	11.11	9.05	13.37	Umesh et al., 2023
Peel	3.94	73.30	3.98	4.20	2.53	12.05	Mala et al., 2024
Core	94.60	4.30	0.60	0.10	0.40	-	Mardawati et al., 2023
Core	6.49	53.83	2.19	3.79	1.47	1.26	Malini et al., 2024
Crown	-	-	0.40	4.20	-	-	Awasthi et al., 2022
Crown	9.25	75.70	5.85	7.61	1.86	-	Brito et al., 2020
Leaves	81.60	-	1.10	-	-	-	Awasthi et al., 2022
Pomace	8.39	31.24	2.33	4.88	2.16	51.00	Meena et al., 2022b
Pomace	4.11	61.81	2.01	5.15	0.84	23.27	Buvaneswaran et al., 2023

anthocyanins, flavanones, flavones, flavonols) and tannins, which is divided into two large groups the condensed and hydrolyzable (Li et al., 2023). Polyphenols have been of great interest in research since their functions benefit human health, this is due to their biological activities such as antioxidant, antimicrobial activity (Valencia-Avilés et al., 2017), antidiabetic and anti-inflammatory (Abbas et al., 2017).

Polyphenols found in pineapple waste

Phenolic Acids

The phenolics acids (PA) they are divided into two groups: hydroxybenzoic acids, which are derivatives of benzoic acid (figure 2a), its structure includes a C6-C1 carbon back bone with methoxylations and hydroxylations in the aromatic ring, and hydroxycinnamic acids; which are derived from cinnamic acid (figure 2b), are characterized by having nine carbons in their structure and a side chain double bond with a cis or trans configuration (Caruso et al., 2022; Kumar and Goel, 2019) The PA they are small molecules that they originate from shikimic and benzoic acids, characterized for having a phenolic ring and a group of acid carboxylic.

Likewise, they are known as secondary metabolite that impart the organoleptic characteristics of foods whether they have sweet or sour flavors (Liu et al., 2020; Rashmi and Negi, 2020; Spiegel et al., 2020). It has been shown that PA have properties important biological properties such as anti-inflammatory, anti-cancer, antimicrobial, antiallergic, antiviral, among others (Kumar & Goel, 2019). The PA common in pineapple waste, ferulic acid has been found, the *p*-hydroxybenzoic acid, chlorogenic acid, *p*-cumaric acid, caffeic acid, , ferulic acid, sinapic acid, in peel and crown (Mala et al., 2024; Sepúlveda et al., 2018).

In the pineapple pulp and waste it has been found, syringic acid, tannic acid, *p*-cumaric acid (Hikal et al., 2021; Segovia-Gómez and Almajano-Pablos, 2016; Vargas-Serna et al., 2022). It has been proven that the stem also contain a variety of phenolics acids, such as gallic acid, chlorogenic acid, *p*-hydroxybenzoic acid, caffeic acid and ferulic acid (Campos et al., 2020). In the pineapple leaves, ferulic acid (Tang and Hassan, 2020).

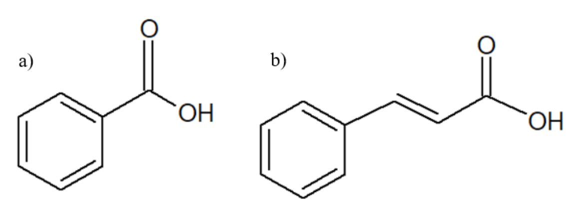
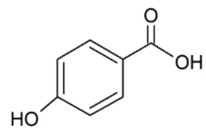
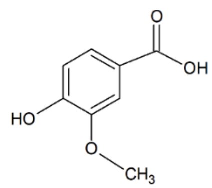
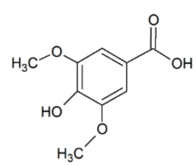
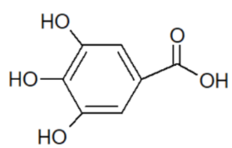
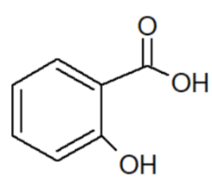
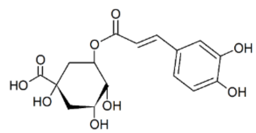
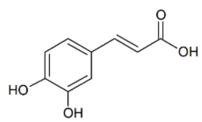
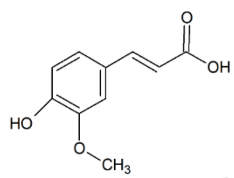
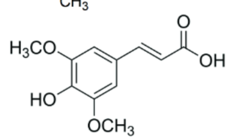


Figure 2. Base structure of the a) hydroxybenzoic and b) hydroxycinnamic acids.

In table 2, you can observe the structures of some PA found in pineapple waste, these have been of great interest since they have various applications both in the food, cosmetic or pharmaceutical industry.

Table 2. Structure of phenolic acids found in pineapple waste (peel, core and crown).

Phenolics acids	Chemical Structure	Applications	Reference
p-Hydroxybenzoic acid (PHBA)		Manufacture of pharmaceutical for brain disease. Improvement in the intestinal microbiota.	(Zhang et al., 2013; Han et al., 2022)
Vanillic acid		Pharmaceutical industry due to its anticancer, anti-inflammatory, antioxidant and other activities.	(Matejczyk et al., 2024)
Syringic acid		Food packaging manufacturing Pharmaceutical potential due to its antioxidant, anti-inflammatory and antitumor activity.	(Huang et al., 2024; Da Silva et al., 2023)
Gallic acid		Edible film manufacturing Used as a dietary supplement	(Bhatia et al., 2022; Xiang et al., 2024; Bilawal et al., 2021)
Salicylic acid		Pharmaceutical potential Potential preservative in food Used as a biostimulator for crops	(Chen et al., 2023a; Chen et al., 2023b)

Chlorogenic acid		Used in pharmaceuticals to combat various diseases Used for skin care	(Nguyen et al., 2024; Rodrigues et al., 2023)
Caffeic acid		Used for skin care Has potential use in combating COVID-19 Potential use in reducing food allergenicity	(Rodrigues et al., 2023; Taysi et al., 2023; Pi et al., 2023)
Ferulic acid		Used in cardiovascular and cerebrovascular diseases Feed for different animal species.	(Zhang et al., 2021; Zheng et al., 2024)
Sinapic acid		Used as a drug, due to its potent biological activities	(Pandi & Kalappan, 2021)

Flavonoids

Flavonoids originate from the phenylalanine and melonyl-CoA pathway. They are composed of a diphenyl-propane backbone containing a benzene ring linked to a pyrone ring and finally to a phenyl ring in position 2 or 3. Flavonoids are classified into various groups, including flavones, flavanols, flavanones, chalcones, anthocyanins, and isoflavones (Figure 3)(Alseekh et al., 2020; Li et al., 2023; Pei et al., 2020).

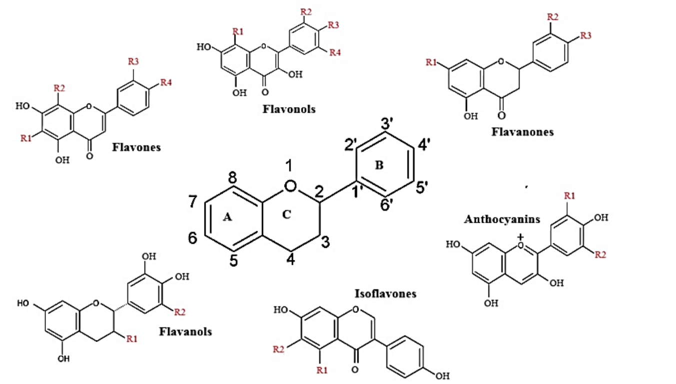


Figure 3. Structure of flavonoids (structures obtained in PubChem, 2025).

Other characteristics of flavonoids are that they may contain glycosidic centers (C) and *O*-glycosides, depending on the union of the sugar (Satari et al., 2021). Currently they have been of great interest, since they have properties beneficial such as a potential antioxidant, antivirals, anti-inflammatory, anticancer, and antibacterial (Dobrzynska et al., 2020; Ravula et al., 2021). It has been proven that flavonoids can be used therapeutically, combating depression, dementia, lupus and even heart disease (Satari et al., 2021).

In another research work studied three types pineapple (smooth cayenne, Tainung 17 and Tainung 19), where using the pulp, peel and stem, for the quantification of flavonoids, the extracts were obtained from 5 g of the waste in 50 mL of ethanol and another extraction with 50 mL of water and kept stirring for 1 h, it was shown that the ethanolic extracts obtained a higher yield than aqueous ones (7.8 to 15.0 µg RE/mg and from 5.64 to 23.5 µg RE/mg, respectively) (Huang et al., 2021).

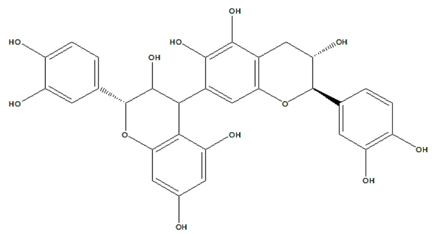
Currently, pineapple waste continues to be studied to be used as food ingredients functional, they made the characterization of pineapple peels to be used as an ingredient for crackers, showed that they contained a total flavonoid concentration of 267.97 mg QE/100 g, and antioxidant activity (Mala et al., 2024). Pineapple residues contain: flavones, isoflavones, flavanols, flavanones, quercetin, quercetin dihydrate, epicatechin gallate, quercetin-3-glucuronide, diosmin, kaempferol and catechin. (Mohsin et al., 2020; Sayago-Ayerdi et al., 2021; Suleria et al., 2020).

Condensed tannins

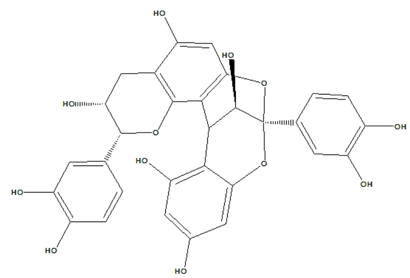
Condensed tannins or proanthocyanidins (Figure 4) are oligomers or polymers of flavan-3-oleyes. Condensed tannins has two homocyclic aromatic rings (A and B) present and a heterocyclic ring (C) (Li et al., 2023; Smeriglio et al., 2017). These compounds have a high molecular weight and can react with aldehydes. In addition to interacting with proteins and carbohydrates through hydrogen bonds (Das et al., 2020; Valencia-Hernandez et al., 2021).

Proanthocyanidins are classified into two types: type A, which are characterized by an additional bond between C2 – C7 of the basic units of condensed tannin and the types B, with additional links between C4 – C4, and although it is not very common also links between C4 – C6 (Smeriglio et al., 2017; Valencia-Hernandez et al., 2021; Watrelot and Norton, 2020). The most common procyanidins include epicatechin and epigallocatechin (Smeriglio et al., 2017). Condensed tannins are the most abundant compared to hydrolyzable tannins; these are generally found in fruits, vegetables, stems, legumes, trees, and flowers (Sharma, 2019).

The condensed tannins have been of great interest since they have antioxidant, antibacterial, antiviral, antiparasitic, anti-inflammatory and antidiarrheal activity (Tong et al., 2022). Others benefits of condensed tannins is that they can reduce blood pressure, as well as improve the coagulation process (Sarkar et al., 2021). A recent study, the solid state fermentation process using *Rhizopus oryzae* was evaluated, the authors concluded that the pineapple peel is a potential source of condensed tannins (Polania-Rivera et al., 2023).



Procyanidin B1



Procyanidin A2

Figure 4. Examples of chemical structure of condensed tannins.

Bromelain

Bromelain (Figure 5) belongs to the group of proteolytic enzymes, being one of the most studied of this group, it is mainly found in plants of the Bromeliaceae family (Varilla et al., 2021). This enzyme is known as protease, which breaks down proteins by cutting the amino acid chains (Chakraborty et al., 2021).

Bromelain include other components such as glycosidases, phosphatases, ribonucleases, cellulases, proxidases and glycoproteases, these are in different proportions, depending on different factors such as the location of the fruit, the soil, weather conditions, extraction method and depending on the part of the pineapple, as well as with a pH of 6 to 7 (stem) and pH 3 to 8 (in the fruit) (Colletti et al., 2021; Hikisz and Bernasinska-Slomczewska, 2021).

The bromelain is still being investigated since it has been found to have important biological properties such as anti-inflammatory, antidiabetic, and anticancer., cardioprotective, immunomodulatory, antioxidants (Hikisz & Bernasinska-Slomczewska, 2021; Kumar et al., 2023). During the pandemic, it was shown that bromelain inhibits the infection of VeroE6 cells by SARS-CoV-2 (Kritis et al., 2020; Tallei et al., 2021). They have been demonstrated that bromelain has beneficial effects in reducing neurotoxicity through antioxidant effects against Alzheimer's (Eraky et al., 2023; Kumar et al., 2022).

It has been proven that bromelain combined with antibiotics more effective in relieving various infections such as rhinosinusitis, bronchitis, pneumonia, sinusitis, infections rectal, of the urinary tract and of the respiratory tract (Locci et al., 2024). The bromelain used in the food, brewing, meat processing, textile, and cosmetic industries because it has low toxicity, is well absorbed by the body, and maintains its biological activity (Hikisz and Bernasinska-Slomczewska, 2021; Varilla et al., 2021).

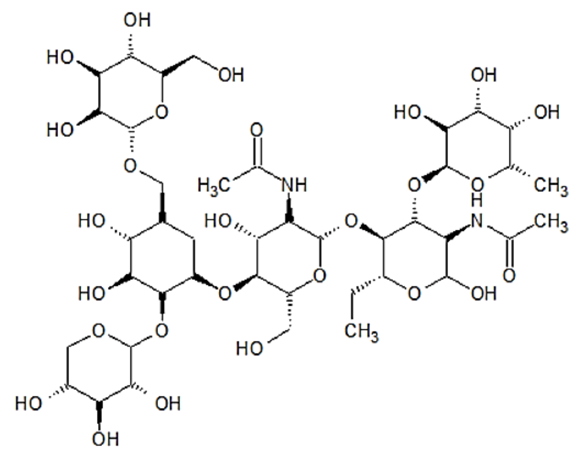


Figure 5. Chemical structure of bromelain.

Biological activities of bioactive compounds found in pineapple waste

Antioxidant activity.

Antioxidant compounds have been of great importance for human health, this is because oxidative stress in cells is related to a wide range of diseases such as cancer, cardiovascular, diabetes or neurodegenerative diseases (Martí-Quijal et al., 2021). The antioxidant capacity is known for its ability to inhibit oxidative degradation, acting mainly against free radicals, due to this the antioxidant compounds are divided according to their reaction mechanism, first are the primary antioxidants, which come from the formation of free radicals; the secondary ones, which inactivate the free radicals already formed and finally the tertiary antioxidants, which repair oxidative damage (Huet-Breña, 2017).

For the compounds to have efficient antioxidant activity, various factors must be taken into account, such as structural properties, temperature or the characteristics of the substrates (Munteanu and Apetrei, 2021). Among the most used tests for determination of antioxidant activity is ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric ion Reducing Antioxidant Power), which are based on the transfer of electrons (Christodoulou et al., 2022).

On the other hand, characterize the bioactive compounds from the peel, core and crown of the pineapple using ultrasound extraction methods The DPPH assay was carried out for the determination of antioxidant activity, giving the best crown extracts were tested at 50% ethanol determined that the 3-methylglutaric acid compounds, threonine, valine and α-linolenic acid, contributed to the antioxidant activity (Azizan et al., 2020). In table 3, some bioactive compounds are observed found in pineapple waste that have activity antioxidant.

Antimicrobial activity

Antimicrobial activity consists of the inhibition of microorganisms that can affect food, vegetables, water, among others (Gunwantrao et al., 2016). Antimicrobial activity is attributed to the chemical interference of bioactive compounds with the synthesis or vital function of the bacteria.

Bioactive compounds, have been studied to use as antimicrobial agents to inhibit bacteria which can grow in the food; as the food is very susceptible to contamination from gram-negative or gram-positive bacteria (Fouda-Mbanga & Tywabi-Ngeva, 2022), such as *Listeria monocytogenes*, *Escherichia coli* (Kumariya et al., 2019), *Pseudomona aeruginosa,* and *Staphylococcus aureus* (Vaou et al., 2021).

Different methods have been studied to determine this activity, among which are disk and well diffusion tests, one of the most used techniques because it is low-cost and simple, and the vial cell counting method, which is generally used to evaluate antimicrobial efficiency (Abdollahzadeh et al., 2021).

Recent studies have shown that extracts of the waste of pineapple are effective to act as antimicrobial agents, extracts contain bioactive compounds that one effective in inhibiting bacterial growth. In table 4 we can see different examples of obtaining extracts from pineapple waste, which have antimicrobial activity. Not only bioactive compounds have proven to be an effective source of antimicrobial effect, but also the characteristic proteolytic enzyme of pineapple (Bromelain) since its non-stick capacity prevents bacteria from adhering to the surface, thanks to the functional groups. which contains bromelain (Agrawal et al., 2022; Gupta, 2022).

Anti-inflammatory activity

The inflammation it is an immune response when an infection or injury occurs in the body, and this is divided into two, the first is the acute one, which occurs immediately after injury and lasts only a few days, while the second is the chronicle and this it is longer lasting (Goh et al., 2022; Moreno-Ley et al., 2021). In turn, is something complex since different processes are involved, such as the migration of leukocytes, activating factor platelet, among others (Oliveira et al., 2022). To carry out the deflating process have been treated mainly with steroidal or non-steroidal anti-inflammatory drugs (Hou et al., 2020).

There is a wide variety of ointments and drugs, however, nowadays the treatments applied for muscular or gastrointestinal inflammation are more natural. For example, some authors studied pineapple peel extracts, demonstrating anti-inflammatory activity significant in reducing exudate formation, inflammatory cell count, and levels of nitrite, tumor necrosis factor alpha, and interleukin-6 (Ajayi et al., 2022). On the other hand, evaluated the process of fermentation in state solid with *Lactobacillus plantarum*, *L. rhamnosus* and *Aspergillus oryzae,* using pineapple peel as a substrate, to obtain bioactive compounds with anti-inflammatory activity. The authors concluded that pineapple extracts are a promising source of compounds with anti-inflammatory activity (Ortega-Hernández et al., 2023).

Table 3. Activity antioxidant of bioactive compounds from pineapple waste.

Part of the pineapple	Extraction method	Main Compounds	Results	Reference
Crown	Solvent extraction	*p*-coumaric acid ferulic acid 4-hydroxybenzaldehyde	DPPH: 244.7; ABTS: 467.8; FRAP: 762.6 µgTE/g	(Brito et al., 2021)
Peel	Soxhlet, supercritical fluids, solvent extraction	Ferulic acid	DPPH: 45%	(Madhumeena et al., 2021)
Peel	Solid state fermentation	Gallic acid Chlorogenic acid Caffeic acid Cinnamic acid	DPPH: 59.76 % ABTS: 73.66 %	(Polania-Rivera et al., 2023)
Peel	Maceration	Catechin Acidvinyl Synaptic acid	DPPH: 2,364.08 mg Trolox/100 g FRAP: 1,365.14 mg $FeSO_4$/100g	(Mala et al., 2024)
Peel and core	Maceration	Total phenolic	DPPH: 34.80–36.45 µmol Trolox.g $^{-1}$ dry matter FRAP: 25.60–27.09 µmol Trolox.g $^{-1}$ dry matter ABTS: 41.43–42.19 µmol Trolox.g $^{-1}$ dry matter dry	(Santos et al., 2021)
Peel Bagasse	Supercritical fluid extraction (SFE) Pressurized liquid extraction (PLE)	Caffeic acid Ferulic acid	SFE: ABTS 0.15-3.38 µM TE/g dw FRAP: 0.85-11.17 µM TE/g dw PLE: ABTS 9.09-57.93 µM TE/g dw FRAP 11.28-56.03 µM TE/g dw	Maia and Fasolin, 2025
Peel	Microwave assisted extraction	Total phenolic Total flavonoid	DPPH: 68-84 % (inhibition)	Harith et al., 2023

Peel	Ultrasound assisted extraction	Gallic acid Syringic acid Caffeic acid Coumaric acid Ferulic acid	ABTS: 0.020 µM Trolox/g DPPH: 0.025 µM Trolox/g	Zampar et al., 2022

Table 4. Pineapple waste extracts with antimicrobial activity.

Pineapple waste	Extraction Method	Compounds	Methodology	Studied Pathogen	Results	Reference
Peel	Maceration, etahanol and methanol extracts	Gallic acid Catechin Epicatechin Ferulic acid Quercetin Kaempferol	Agar diffusion (at two extract concentrations, 75 mg/mL and 40 mg/mL) Minimum Inhibitory Concentration (MIC)	*Bacillus cereus, S. aureus, E. coli, Salmonella typhimurium*	The highest zone of inhibition at 75 mg/mL was obtained against *E. coli*, reaching ± 21.0 mm. MIC: 0.62 mg/ml methanol extracts and 1.25 mg/mL ethanol extracts against *B. cereus*.	(Jatav et al., 2022**)**
Peel	Maceration, methanol extracts of 70%	Flavonoids, tannins, alkaloids and phenols	Agar diffusion, with different concentrations of extract (0 ppm, 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm)	*B. subtilis*, *E. coli* and *Candida albicans*	The 2000 ppm conc. showed the highest percentage against *C. albicans* (7.5 ± 0.17 mm)	(Dewi and Simamora, 2023)
Peel Stem Heart	Maceration extracts with distilled water	-	Agar diffusion Minimum Inhibitory Concentration (MIC)	*Aeromonas hydrophila*	Inhibition zone: 7.96 ± 0.20 mm and MIC: 125 mm/L	(Khumsrisuk et al., 2022)
Peel and crown	Solid-state fermentation	Chloretin 2'-*O*-xylosyl-glucoside Feruloyl tartaric acid	Minimum Inhibitory Concentration (MIC)	*S. aureus* and *L. monocytogenes*	Effective against *S. aureus* and *L. monocytogenes*, with MIC: 50 mg/mL and 12.5 mg/mL, respectively	(Paz-Arteaga et al., 2023a)

Peel	Soxhlet (extracts with, petroleum ether, ethyl acetone, etganol water, tetracycline y DMSO)	-	Agar diffusion	*S. aureus, E. coli P. aeruginosa , Vibrio cholera Klebsiella pneumoniae*	*P. aeruginosa with* tetracycline (24 ± 0.15 mm) *S. aureus* with ethyl acetate (16 ± 0.08 mm) *E. coli* with ethyl acetate (20 ± 0.12 mm) *V. cholerae* with ethyl acetate (20 ± 0.12 mm) *K. pneumoniae* with ethyl acetate (16 ± 0.08 mm)	(Lubaina et al., 2019)
Peel, stem, peel core and crown	Maceration (extracts with sodium acetate buffer)	Bromelain	Minimum Inhibitory Concentration (MIC)	*S. aureus* and *P. acne*	*P. acne* was found susceptible against the purified fractionates at concentrations as low as 19 lg/ml of the fruit extract	(Abbas et al., 2021)

Not only the extracts methanolic or ethanolic have been shown to be effective for significant inflammatory activity, but bromelain has also been shown to have anti-inflammatory properties. However, the molecular mechanisms underlying its anti-inflammatory effects are not well understood. Some authors investigated the effects of crude and purified bromelain on lipopolysaccharide-induced inflammation in macrophages RAW 264.7. The bromelain pretreatment reduced proinflammatory cytokines and mediators, which were related to the downregulation of iNOS and COX-2 expressions (Insuan et al., 2021). Another research evaluated the anti-inflammatory activity of bromelain from pineapple stem powder in a cream preparation. It observed its in vitro stability, optimizing three different cream formulas to determine the most stable one. The anti-inflammatory test showed a stable concentration of 34.41%, 32.06% and 37.82%, indicating that the pineapple stem powder has moderate anti-inflammatory activity (Masbagusdanta et al., 2020).

Anticancer activity

Cancer is one of the diseases furthermore such throughout the world, this is mainly due to genetic mutations as well as various anticancer agents that influence cell functions (Kurniawan et al., 2021). Treatments against cancer are generally divided into pharmacological therapy and non-pharmacological therapy, such as chemotherapy. However, it has several adverse effects (Chen et al., 2020; Kciuk et al., 2023).

That is why they are looking for other alternatives such as bioactive compounds, since it has been shown that various compounds help fight various types of cancer. It has been shown that the bromelain and flavonoids that contain pineapple, have anticancer activity by inducing apoptosis in cancer cells through p53 induction mechanisms, upregulation of Bcl-2, which is an antiapoptotic protein, upregulation of Bax, which is a proapoptotic protein, induction of caspases, decreases the expression of COX2 and regulates the MAPK and Akt/PKB pathway to inhibit the NF-kB pathway (Nida & Haryoto, 2022).

The effectiveness of bromelain (extracted from pineapple peel and crown) was evaluated against two breast cancer cell lines (MDA-MD 231 and MCF-7), in addition to evaluating the anti-inflammatory effect (against the COX-2 gene). The results of the present article indicate that bromelain (at a concentration of 200 µg/ml), had a significant effect in reducing cell viability in the cancer lines studied; furthermore, it was observed that bromelain induced apoptosis and reduced the expression of the COX-2 gene, suggesting its potential as an anticancer and anti-inflammatory agent (Mohamad & Sedrah, 2023).

Antidepressant activity

Depression is a global health disease which affects one in ten people; this disease can affect behavior, motivation, thoughts, feelings, among other emotional factors (Chen et al., 2022; Singh et al., 2021). Depression is associated with oxidative stress levels, since if there is an excess of these molecules, it produces damage causing nervous inflammations, thus causing depressive states (Black et al., 2015).

Currently, different drugs (fluoxetine, sertraline, paroxetine among others) have been used to combat depression, however, the drugs have been found to have undesirable side effects (Rakib et al., 2020). Therefore, new alternatives are sought for the creation of new antidepressants, which do not become harmful to health, in a study it was demonstrated that methanolic extracts of pineapple peel are effective in combating depression, reducing the inactivity time of rats during tail suspension tests. Dosing (3.25, 7.5 and 15 mg/kg) produced an increase in active behavior, suggesting improved motivation and reduced despair, thus promoting healthier alternatives to traditional medications (Kafeel et al., 2016).

In a more recent study, the effect of pineapple (*Ananas comosus*) pulp on lipopolysaccharide (LPS)-induced depressive behaviors in rats was investigated. Pineapple pulp was administered for 28 days prior to LPS injection, resulting in a reduction in immobility time and an increase in serotonin levels. The findings suggest that pineapple pulp may have antidepressant properties by enhancing tryptophan availability (Nurrahma et al., 2024).

CONCLUSION

This document highlights the valorization of pineapple waste, emphasizing its richness in bioactive compounds such as polyphenols, tannins, and bromelain. These compounds possess diverse biological activities, including antioxidant, antimicrobial, anti-inflammatory, anticancer, and antidepressant properties. The review highlights the potential of these compounds in the prevention of chronic diseases and their application in the pharmaceutical, food, and cosmetic industries. This work lays a solid foundation for future research into the characterization and use of these bioactive compounds in the development of natural therapies and functional products.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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Impacto del Método de Extracción sobre las Propiedades Fisicoquímicas, Reológicas y Floculantes de un Biofloculante de *Aloe vera*

Impact of the Extraction Method on the Physicochemical, Rheological and Flocculating Properties of an *Aloe vera* Bioflocculant

Juarez-Sánchez, J.A.,[1] Bustos-Ramírez, K.,[1] Varela-Santos, E.C.,[1] Avendaño-Vasquez, G.,[1] Vargas- García, K.A.[1*]

[1]*TecNM/ITS de Tierra Blanca-Maestría en Ciencias de los Alimentos y Biotecnología, Ave. Veracruz S/N, Col. PEMEX, C.P.95180. Tierra Blanca, Veracruz. México*

**Corresponding Author: k.vargas@itstb.edu.mx*

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Resumen

La contaminación en cuerpos de agua requiere alternativas de tratamiento sostenibles. El uso de biofloculantes de *Aloe vera* que permitan sustituir floculantes sintéticos fue evaluado. La materia prima se recolectó de Tierra Blanca, Veracruz, México; y la aloína se removió por inmersión (agua, 48 h). El gel se separó, cortó, trituró y homogeneizó. La extracción por maceración se llevó primero en inmersión con agua (relación S/L 1:1, 24 h) y posteriormente con etanol (96 %, relación S/L 1:3, 24 h). Mientras que la extracción por microondas con etanol (96 %, relación S/L 1:3, 5 min con pulsos de 0.5 min y 800 W). El biofloculante extraído fue secado (45 °C, 6 h) y triturado. La caracterización fisicoquímica del gel y biofloculante se realizó considerando humedad, sólidos totales, °Brix, pH, azúcares reductores, carbohidratos totales, ácido málico; en tanto que el desempeño se realizó en términos de propiedades reológicas (viscosidad, índice de consistencia y comportamiento de flujo) y reducción de turbidez (NTU). El mayor contenido de sólidos solubles, ácido málico, ºBrix, azúcares reductores y carbohidratos totales se observó en el biofloculante. El gel y biofloculante mostraron un comportamiento no Newtoniano, tipo pseudoplástico con el modelo de Otswald (R^2 >0.99, y n <1) y la mayor reducción de turbidez se observó a pH 7.5 tanto en gel y biofloculante (78.31 %). Todo lo anterior independientemente del método de extracción. La extracción por microondas requiere menor tiempo de obtención del biofloculante de *A. vera*, pero no tuvo efecto sobre sus propiedades fisicoquímicas, estructurales, reológicas y floculantes, representando una alternativa sostenible y ecológica para sustituir floculantes sintéticos.

Palabras clave: Floculantes; Polisacáridos; Sábila; Tratamiento de aguas; Turbidez.

Abstract

Water pollution requires sustainable treatment alternatives. The use of *Aloe vera* bioflocculants to replace synthetic flocculants was evaluated. The raw material was collected from Tierra Blanca, Veracruz, Mexico; and the aloin was removed by immersion (water, 48 h). The gel was separated, cut, crushed and homogenized. Extraction by maceration was first carried out by immersion with water (S/L ratio 1:1, 24 h) and then with ethanol (96 %, S/L ratio 1:3, 24 h). While microwave extraction with ethanol (96 %, S/L ratio 1:3, 5min with 0.5 min pulses and 800 W). The extracted bioflocculant was dried (45 °C, 6 h) and grinded. The physicochemical characterization of the gel and bioflocculant was carried out considering moisture, total solids, °Brix, pH, reducing sugars, total carbohydrates, malic acid; the performance was done in terms of rheological properties (viscosity, consistency index and flow behavior) and turbidity reduction (NTU). The highest content of soluble solids, malic acid, ºBrix, reducing sugars and total carbohydrates was observed in the bioflocculant. The gel and bioflocculant showed a non-Newtonian pseudoplastic behavior with the Otswald model (R^2 >0.99, and n <1) and the greatest reduction in turbidity was observed at pH 7.5 in both gel and bioflocculant (78.31 %). The above regardless of the extraction method, microwave extraction requires less time to obtain the biofloculant *A. vera*, but had no effect on its physico-chemical, structural, rheological and flocculating properties, representing a sustainable and ecological alternative for replacing synthetic flocculants.

Keywords: Flocculants; Polysaccharides; Sabila; Water treatment; Turbidity.

INTRODUCCIÓN

La contaminación por descargas sobre cuerpos de agua y el deficiente funcionamiento de las plantas de tratamiento han ocasionado un problema socioambiental en el Estado de Veracruz, México. Este estado es abundante en plantas campestres; ocupando el segundo lugar de producción nacional de la sábila, cuyo nombre científico es *Aloe vera* L. (SIAP 2017). Esta planta (Figura 1) es de propagación sencilla para climas secos (Lagunes-Dominguez et al., 2024), y es abundante en el municipio de Tierra Blanca, Veracruz, donde su desarrollo y reproducción ocurre de manera natural, sin embargo, en la actualidad se le han dado muy pocas aplicaciones. El *A. vera* contiene polisacáridos entre sus principales compuestos bioactivos (por ejemplo el acemanano, Ac.), los cuales poseen excelente degradabilidad y biocompatibilidad, son considerados biomateriales de alto valor con amplio potencial de aplicación en procesos tecnológicos sostenibles para el área ambiental, alimentaria y farmacológica; y desempeñan un papel clave en las propiedades floculantes del *A. vera* (Bai et al., 2023).

Figura 1. Planta de sábila (*A. vera*)

La capacidad de floculación del *A. vera* se ha atribuido a la presencia de polisacáridos hidrosolubles como componentes principales del gel, que poseen grupos funcionales (por ejemplo grupos carboxilos e hidroxilos) con la capacidad de flocular partículas al interactuar con contaminantes y sólidos suspendidos desestabilizando las suspensiones coloidales (Katubi et al., 2021; Figueiredo et al., 2022; Venegas-García et al., 2024). Los biofloculantes son sustancias orgánicas de que facilitan la agregación de partículas suspendidas en el agua, permitiendo su posterior sedimentación, eliminación y clarificación; y abordar los desafíos ambientales que plantea la contaminación de las aguas residuales y prevenir el ingreso de contaminantes químicos nocivos en la cadena alimentaria (Thamaraiselvi et al., 2024). Por lo que su uso representa una alternativa sostenible en sistemas de tratamiento de agua para mitigar el impacto socio ambiental ocasionado por la descarga inmoderada, y la subsecuente acumulación de sólidos suspendidos, residuos tóxicos y metales pesados (Das et al., 2021, Arcila & Peralta, 2016; Giannakoudakis et al., 2018; Diestra & Ramos 2019). Adicionalmente, Valdivia-Rivera et al. (2018), indicaron que la tasa de enfermedad renal crónica (ERC) en el municipio de Tierra Blanca, Veracruz está relacionada principalmente por el agua dulce que es de consumo humano, y que está contaminada por hidrocarburos, pesticidas, entre otros. Considerando lo anterior, el aprovechamiento de *A. vera* podría utilizarse para desarrollar procesos tecnológicos, que permitan sustituir floculantes sintéticos, reducir contaminantes químicos en cuerpos de agua y suelos de esta región; así como minimizar la acumulación de residuos tóxicos en el ambiente, ofreciendo una alternativa más sostenible y ecológica en diversas aplicaciones industriales. Lo anterior promoviendo la sustentabilidad en el manejo de recursos naturales a la economía circular y principalmente en el saneamiento de las aguas residuales del municipio de Tierra Blanca, Veracruz. Resultando de interés, evaluar el impacto del método de extracción sobre las propiedades fisicoquímicas, reológicas y floculantes de un biofloculante de *A. vera*; para desarrollar una alternativa de obtención de floculantes naturales a partir de la sábila de la región de Tierra Blanca, Ver.

MATERIALES Y MÉTODOS

Recolección de materia prima

La materia prima (sábila) se recolectó en tres zonas del municipio de Tierra Blanca, Veracruz: Col. La Floresta, Col. Pemex y Col. Barrio de Torreón. Las hojas de la sábila fueron colectadas en dos períodos estacionales (abril-mayo, y octubre-noviembre) evitando la temporada de lluvia en el municipio. Se tomaron las hojas inferiores en forma angular evitando el daño en las hojas superiores (Lagunes-Domínguez et al, 2024). Posteriormente las hojas fueron lavadas para la remoción de residuos, y colocadas en inmersión con exceso de agua para la remoción de la aloína residual (48 h). En la Figura 2, podemos observar los mapas geográficos de las zonas de recolección de la materia prima y que son acorde al impacto socioambiental de la presente propuesta.

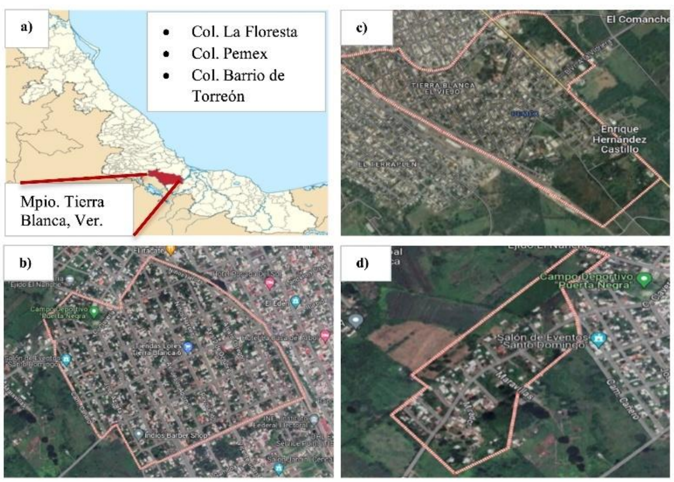


Figura 2. Zonas de recolección de la materia prima. a) Zona de influencia del impacto social, b) Col. Barrio de Torreón, c) Col. Pemex, d) Col. La Floresta.

Extracción del biofloculante

Una vez removida la aloína, la corteza y las espinas de las hojas del *A. vera*, el gel se separó, cortó, trituró y homogeneizó. La extracción por maceración se llevó a cabo en dos etapas de acuerdo al método reportado por Otálora et al. (2021) donde en la primera etapa se realizó una inmersión con agua con una modificación en la relación sólido: líquido (S/L 1:1, 24 h), posteriormente el gel hidratado fue filtrado y llevado a una segunda etapa donde se sometió a maceración por inmersión con etanol (96 %, relación S/L 1:3, 24 h). Mientras que en la extracción por microondas el gel fue hidratado solo durante 2 h con agua (S/L 1:1, 24 h), y posteriormente el gel hidratado fue filtrado y se sometió a extracción con etanol (96 %, relación S/L 1:3, 5 min con pulsos de 0.5 min y 800 W) acorde a lo reportado por Salomón-Izquierdo et al. (2022). El biofloculante extraído fue secado en estufa 9023A ECOSHEL (45 °C, 6 h) y triturado independientemente del método de extracción evaluado. Todas las extracciones se realizaron por triplicado.

Caracterización fisicoquímica y estructural

La caracterización fisicoquímica y estructural se realizó tanto en el gel del *A. vera* (fresco) como en los biofloculantes obtenidos en estado seco (polvo). La caracterización fisicoquímica se llevó a cabo en términos de humedad, sólidos totales, cenizas, °Brix, pH, y ácidez (% ácido málico). En el caso de humedad, sólidos totales y cenizas, estas determinaciones se realizaron gravimétricamente por pérdida de masa de las muestras sin dilución (AOAC, 2000), utilizando una estufa de secado 9023A ECOSHEL a (100 °C, 24 h), y una mufla Lab Tech LEF-205S-0 (540 °C, 4 h). Mientras que la determinación de los Solidos Solubles Totales (ºBrix) y el pH se llevaron a cabo por dilución másica del gel de *A. vera* y de los biofloculantes con agua destilada (1:2), empleando un refractómetro análogo Bellingham + Stanley Eclipse, modelo 45-05, y un potenciómetro OHAUS AB23PH-F S calibrado a 3 puntos con soluciones estándar de pH 4, 7 y 10 respectivamente (AOAC, 2000). La obtención de ácido málico, se realizó sobre diluciones másicas de las muestras del gel de *A. vera* y de los biofloculantes con agua destilada (1:25) por volumetría acido-base, utilizando NaOH (0.1 N) como agente valorante, fenolftaleína como indicador y aplicando el factor para acido málico (AOAC, 2000). Mientras que la caracterización estructural se realizó en términos de la concentración de azúcares reductores y polisacáridos totales por espectrofotometría UV-VIS, con una adaptación de los métodos reportados por Miller (1959) y DuBois et al. (1956), utilizando una curva calibración de glucosa (0.1-1.0 g/L y 2.0-20.0 µg/mL respectivamente) y un espectrofotómetro UV-VIS (Thermo Scientific Genesys 10s) a 540 nm y 485 nm respectivamente. La concentración de las muestras del gel de *A. vera* y de los biofloculantes, se obtuvo de acuerdo a la ecuación de la recta de la curva calibración de glucosa y el factor de dilución de las muestras preparadas del gel de *A. vera* y biofloculantes con agua destilada. La identificación cualitativa de pectina y ácidos carboxílicos se realizó por dilución másica de la muestras del gel de *A. vera* y de los biofloculantes con agua destilada (1:10) con adición de etanol acidificado al 0.01 % V/V (relación L:L 1:3) para la prueba de pectina (Davitadze, 2023); y (1:4) con bicarbonato de sodio al 10 % P/P (Guarnizo & Martínez, 2009). Donde la respuesta positiva está relacionada con la precipitación de polisacáridos de alto peso molecular para la prueba de pectina, y la efervescencia como evidencia de la liberación del CO_2, derivado de la presencia de los grupos carboxílicos. La caracterización fisicoquímica y estructural se realizó en el gel de *A. vera*, y en los biofloculantes obtenidos por maceración y por microondas. Todas las determinaciones se realizaron por triplicado.

Caracterización reológica

La caracterización reológica del gel del *A. vera* (fresco) y de los biofloculantes obtenidos en estado seco (polvo), se realizó en términos de viscosidad, índice de consistencia y comportamiento de flujo. Estas propiedades reológicas se determinaron utilizando el gel de *A. vera* (fresco) sin dilución y con los biofloculantes utilizando diluciones másicas con agua destilada (1:50); y como testigos se utilizaron glicerina (≥ 99 %) y sorbitol (≥ 91 %) sin dilución. La caracterización reológica se realizó utilizando un viscosímetro rotacional POLYVISC® FV, con la configuración de spindel R2 y con velocidades de rotación entre 0.3 rpm (1.6 s^{-1}) y 100 rpm (542 s^{-1}). El volumen de operación de los fluidos fue de 300 mL y la temperatura de la muestra fue monitoreada y controlada a 25 ºC. Las lecturas correspondientes a la viscosidad de los fluidos se tomaron con variación en las velocidades de corte correspondientes a las velocidades de rotación (Chin et al., 2009; Jorge et al., 2018). Los modelos matemáticos empleados para la determinación de propiedades reológicas y el ajuste de los datos experimentales fueron los modelos de Ley de Newton (Ec. 1) y de Ostwald o de Ley de la Potencia (Ec. 2). Todas las mediciones se realizaron por triplicado.

Ec. 1 $\tau = \mu\gamma$

Ec. 2 $\tau = K\gamma^{n}$

Donde τ corresponde al esfuerzo cortante ($N.m^2$, Pa), μ coeficiente de viscosidad (Pa.s, mPa.s), γ velocidad de corte (s^{-1}), K Indice de consistencia, *n* índice de comportamiento de flujo ($Pa.s^n$).

Desempeño de los biofloculantes

El desempeño de los floculantes se evaluó en términos de reducción de turbidez (NTU), con el gel de *A. vera* (fresco), con los biofloculantes obtenidos por maceración y microondas en estado seco (polvo), y con sulfato de aluminio ($Al_2(SO_4)_3$) como floculante comercial. Lo anterior se llevó a cabo con cinéticas de sedimentación destructivas con un sistema modelo. El sistema modelo se preparó con caolín (polvo) y agua destilada (300 rpm, 30 min) para obtener la turbidez deseada (1000 NTU), donde el pH se ajustó entre 5.5 a 8.0 con HCl y/o NaOH (0.1 N) (Al-Sameraiy, 2024). Las dosis empleada fue de 25 mL para todos los floculantes evaluados: Sulfato de aluminio (10 gL^{-1}), gel de *A. vera* (100 gL^{-1}), biofloculantes obtenidos por maceración y microondas (10 gL^{-1}). La reducción de turbidez (NTU) se determinó por espectrofotometría UV-VIS, con una adaptación del método NMX-AA-038-SCFI-2001, utilizando una curva calibración de Formacina ($C_6H_{12}N_4$) y un espectrofotómetro UV-VIS (Thermo Scientific Genesys 10s) a 420 nm. A partir de la curva calibración y la medición experimental del sobrenadante, se determinó la remoción de la turbidez del sistema modelo en cada cinética con los floculantes evaluados (Ec. 3). Todas las muestras se realizaron por triplicado.

Ec. 3 $\%\,Reducción\,(NTU) = \left[\frac{T_O - T_F}{T_O}\right] \text{x } 100$

Donde T_O corresponde a la Turbidez inicial de la solución modelo (NTU), T_F a la Turbidez en el tiempo final (NTU) de la cinética de sedimentación. La estrategia experimental hasta ahora descrita se muestra en la figura 3.

Análisis estadístico

El efecto del método de extracción sobre las propiedades fisicoquímicas y estructurales del biofloculante de *A. vera* se analizó con un diseño factorial de una sola vía con tres niveles. El factor (método de extracción) y los niveles codificados se muestran en la tabla 1. La experimentación fue realizada por triplicado sobre diez variables de respuesta: humedad, sólidos totales, cenizas, °Brix, pH, ácido málico, azúcares reductores, polisacáridos totales, pectina y ácidos carboxílicos con un total de 9 corridas experimentales.

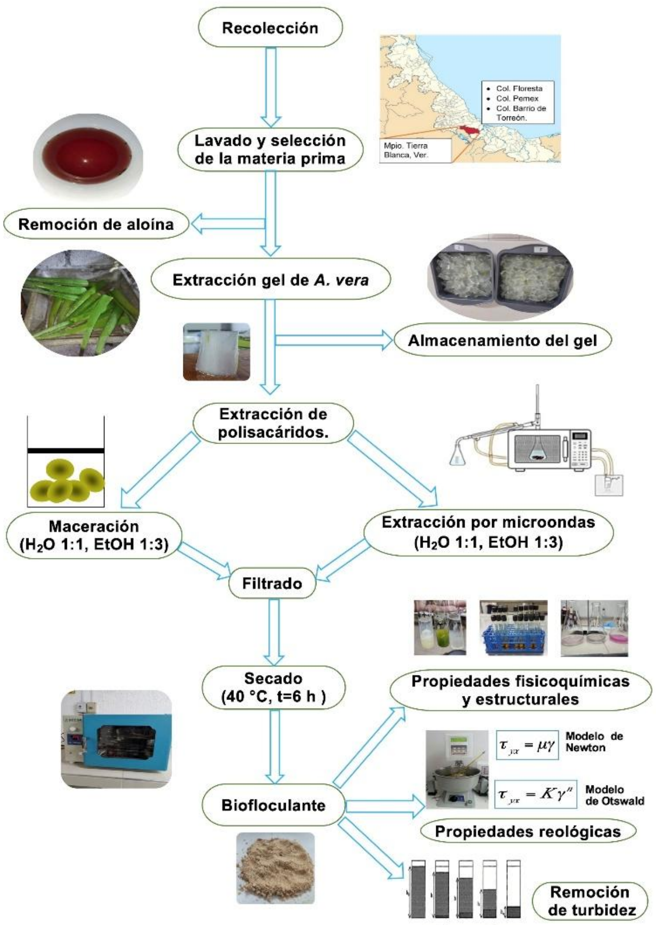


Figura 3. Estrategia experimental en la obtención de biofloculantes de *A. vera*

Adicionalmente el desempeño del biofloculante de *A. vera* se analizó con un diseño factorial con dos factores (x): tipo de floculante (x_1) y pH (x_2), a cuatro y seis niveles respectivamente. Los factores y niveles codificados se muestran en la tabla 2. La experimentación fue realizada por triplicado teniendo como variable de respuesta el porcentaje en la reducción de turbidez (NTU) con un total de 72 corridas experimentales.

Tabla 1. Factores, niveles codificados y valores utilizados en el diseño de experimentos para evaluar el efecto del método de extracción sobre las propiedades fisicoquímicas

Factor	Valores de los niveles codificados		
(x_1)	-1	0	+1
Método de Extracción	Sin Extracción	Maceración	Microondas

El análisis estadístico para ambos diseños fue realizado aplicando el software Design-Expert versión 7.1 mediante un análisis de ANOVA con un nivel de confianza del 95 %.

Tabla 2. Factores, niveles codificados y valores utilizados en el diseño de experimentos para evaluar el efecto del método de extracción sobre el desempeño del biofloculante de *A. vera*.

Factor (Símbolo)		Valores de los niveles codificados
Tipo de Floculante (x_1)	**pH (x_2)**	
No Aplica	5.5	-3
$Al_2(SO_4)_3$	6.0	-2
Gel de *A. vera*	6.5	-1
Biofloculante *A. vera* por maceración	7.0	+1
Biofloculante *A. vera* por microondas	7.5	+2
No Aplica	8.0	+3

RESULTADOS Y DISCUSIÓN

Caracterización fisicoquímica y estructural

La Tabla 3 muestra los resultados de la caracterización fisicoquímicas del gel y de los biofloculantes de *A. vera*. Los resultados indican la mayor presencia de sólidos totales, ºBrix, ácido málico, azúcares reductores y carbohidratos totales en los biofloculantes independientemente del método de extracción evaluado, mientras que los valores de humedad, cenizas y pH fueron mayores en el gel de *A. vera*. La diferencia de los valores obtenidos para el caso de azúcares reductores y carbohidratos totales en el gel se atribuyó a que la determinación se realizó en base húmeda (Perez-Benites, 2019). La presencia de ácidos carboxílicos y de pectina fue identificada tanto en el gel como en los biofloculantes del *A. vera* independientemente del método de extracción evaluado. Los resultados del análisis estadístico indicaron que no existe diferencia significativa entre las propiedades fisicoquímicas, ni entre las propiedades estructurales de los biofloculantes obtenidos por maceración y por microondas; pero si, con las propiedades fisicoquímicas y estructurales del gel de *A. vera* ($p < 0.05$), con excepción de la pruebas de pectina y de bicarbonato. Esto ultimo confirmó la conservación de polisacáridos y de grupos carboxílicos en los biofloculantes obtenidos por ambos métodos, y sugiere propiedades floculantes similares a las del gel de *A. vera* (Katubi et al., 2021; Figueiredo et al., 2022; Venegas-García et al., 2024).

Adicionalmente, una ventaja del proceso de extracción del biofloculante por microondas es el tiempo de proceso (2 h y 5 min en total) en comparación con el proceso de maceración (48 h en total).

Caracterización reológica

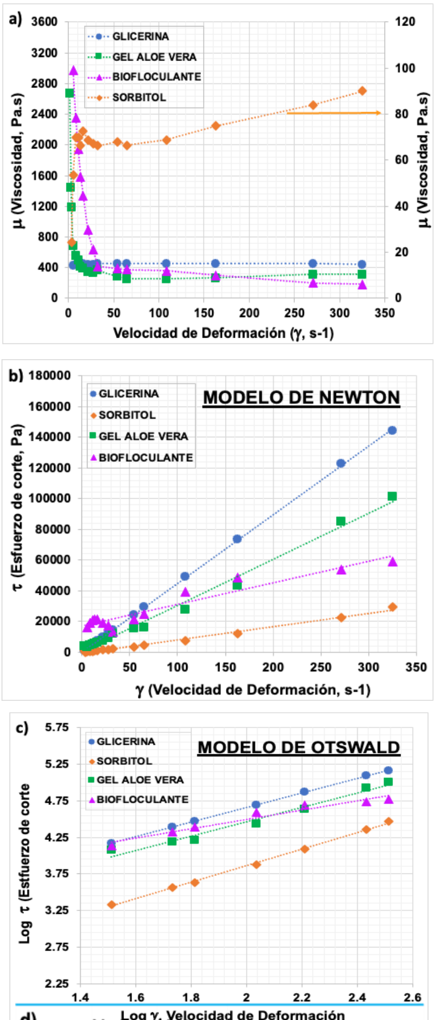
Por otra parte, en la Figura 4 se muestran las propiedades reológicas y los perfiles de flujo del gel de *A. vera*, del biofloculante y de los fluidos testigos (glicerina y sorbitol).

Tabla 3. Resumen de la caracterización fisicoquímica y estructural del gel de *A. vera* y los biofloculantes obtenidos por maceración y microondas.

Propiedad (g/100g)	**Gel (*A. vera*)**	**Biofloculante (Maceración)**	**Biofloculante (Microondas)**
Humedad	98.7 ± 0.36^a	11.43 ± 0.83^b	12.65 ± 1.13^b
Sólidos totales	1.50 ± 0.36^a	88.56 ± 0.83^b	87.35 ± 1.13^b
Cenizas	5.27 ± 0.01^a	2.62 ± 0.86^b	2.71 ± 0.93^b
SST (°Brix)	2.00 ± 0.00^a	5.00 ± 0.00^b	5.00 ± 0.50^b
pH[1]	5.52 ± 0.15^a	4.83 ± 0.52^b	4.81 ± 0.31^b
Ácido málico	2.81 ± 0.35^a	187.86 ± 7.33^b	187.63 ± 4.22^b
Carbohidratos totales	4.44 ± 0.01^a	25.34 ± 1.57^b	24.34 ± 1.65^b
Azúcares reductores	7.37 ± 0.41^a	35.16 ± 1.70^b	35.87 ± 1.28^b
Pectina[2]	$+++^a$	$+++^a$	$+++^a$
Acidos carboxílicos[2]	$+++^a$	$+++^a$	$+++^a$

Notas: [1]Valores de 1- 14; [2]Cualitativa: Positiva (+++) ó negativa (---). Letras diferentes en la misma fila expresan que existen diferencias significativas ($p<0.05$).

Puede observarse en esta figura el comportamiento del tipo Newtoniano para la glicerina al presentar una viscosidad constante independientemente de la velocidad de deformación, mientras que, para el sorbitol, el gel y el biofloculante de *A. vera* se observa un comportamiento del tipo no Newtoniano. El perfil de flujo de la glicerina confirmó su comportamiento Newtoniano al mostrar el esfuerzo cortante como una función lineal de la velocidad de corte acorde a los fluidos Newtonianos, y un mayor ajuste de los datos experimentales con el Modelo de Newton ($R^2 > 0.99$). Mientras que, para el sorbitol, el gel y los biofloculantes de *A. vera* puede verse que el esfuerzo cortante no es una función lineal de la velocidad de corte, sugiriendo nuevamente un comportamiento no Newtoniano (Figura 4).



d)

Fluido	Modelo Newton (R^2)	Modelo Ostwald (R^2)	Indice de comp. de Flujo(n)	Tipo de fluido
Glicerina	0.999	0.999	1.00	Newtoniano
Sorbitol	----	0.996	1.143	No Newtoniano Dilatante
Gel de *A. vera*	----	0.975	0.814	No Newtoniano Pseudoplástico
Biofloc. de *A. vera*	----	0.954	0.652	No Newtoniano Pseudoplastico

Figura 4. Caracterización reológica. a) Curvas de Viscosidad Vs velocidad de deformación. b) Curvas de Flujo Modelo de Newton. c) Curvas de Flujo Modelo de Otswald o Ley de Potencia. d) Resumen de las propiedades reológicas.

También puede observarse en la Figura 4 que el perfil de flujo del sorbitol, del gel y del biofloculante de *A. vera*, confirmó su comportamiento no Newtoniano, donde los datos experimentales presentaron el mejor ajuste con el Modelo de Otswald o Ley de la Potencia (R^2 >0.95); así mismo los valores de índice de comportamiento de flujo confirmaron un comportamiento de fluido no Newtoniano del tipo dilatante (n >1) para el sorbitol, y un comportamiento de fluido no Newtoniano del tipo pseudoplástico (n >1) tanto para el gel como para el biofloculante de *A. vera* (Figura 4). Los valores de índice de comportamiento de flujo reportados por Sonawe et al. (2020) (n = 0.48 - 0.55) coinciden con los obtenidos en este estudio, lo que confirma un comportamiento reológico similar en el gel de *A. vera*. Este resultado es un indicativo indirecto de la conservación del acemanano tanto en el gel como en el biofloculante obtenido independientemente del método de extracción evaluado; ya que el comportamiento adelgazante al corte está relacionado con sus grupos acetilos; mientras que un comportamiento del tipo Newtoniano en los derivados o productos del gel de *A. vera* indican la descomposición de este polisacárido como una consecuencia del almacenamiento y manejo inadecuado del gel; así como a las condiciones de proceso, en este caso del biofloculante (Sonawane et al., 2020; Bai et al., 2023). Adicionalmente se confirmó la aplicabilidad del Modelo de Newton y Ley de la Potencia para la caracterización reológica del gel de *A. vera* y su biofloculante, lo cual coincide con lo reportado previamente no sólo para *A. vera*, sino también para productos naturales y formulados (hidrocoloides, jugo de tomate, jugos citricos, jugo de caña) que contienen polisacáridos como compuestos principales (Chin et al., 2009: Astolfi-Filho et al., 2011; Jorge et al., 2018; Sonawane et al., 2020). Además el conocimiento de propiedades reológicas está relacionada con la calidad del producto, su estabilidad en etapas de procesamiento almacenamiento y consumo; así como con el diseño, optimización y aplicación en procesos tecnológicos para este tipo de productos. Al respecto este trabajo puntualiza la importancia del comportamiento reológico en el gel y en el biofloculante de *A. vera*, ya que podría ser un parámetro critico durante su aplicación en sistemas de tratamiento de agua, y cuya vertiente ha sido poco explorada a diferencia del área alimentaria (Diestra & Ramos 2019).

Desempeño de los biofloculantes

En la Tabla 4 se muestra la matriz experimental y los resultados obtenidos para la variable de respuesta estudiada en el desempeño de los floculantes, donde los porcentajes de reducción de la turbidez más altos se observan a valores de pH de 7.5 tanto con gel como con el biofloculante de *A. vera*, independientemente del método de extracción evaluado, en comparación con el floculante comercial (Sulfato de aluminio). Estos resultados coinciden con lo reportado por Adco et al. (2024), con condiciones de operación y dosis similares de gel de *A. vera* (79.36 % de reducción de turbidez), pero con una dosis mayor a la del biofloculante y una turbidez inicial 10 veces menor a la del presente trabajo (110 NTU).

Benalia et al. (2021), evaluaron el desempeño del gel y del biofloculante de *A. vera* en los estados físicos similares al del presente trabajo (gel en fresco y biofloculante en polvo) con remociones de turbidez de 87.84 % y 28.23 % respectivamente; sin embargo, la diferencia en porcentajes de remoción para el caso del biofloculante en polvo se atribuyó a una menor dosis empleada en comparación al presente trabajo. Además, el valor inicial de turbidez fue 100 veces menor (13 NTU) al del presente trabajo.

Por otro lado, en la tabla 5 se muestra el análisis de varianza ANOVA del efecto del método de extracción y del pH sobre sobre el desempeño de los floculantes. Puede verse en esta tabla que el efecto tanto del tipo de floculante, como del pH, y la interacción de estos dos factores (x_1, x_2 y x_1x_2 respectivamente) fue significativo estadisticamente sobre el porcentaje de reducción de turbidez (NTU) al 95 % de nivel de confianza.

Tabla 5. Tabla de analisis de varianza ANOVA del efecto del tipo de floculante y del pH sobre sobre el desempeño de los floculantes

Términos del modelo	GL	SC	F	*p*
x_1	3	292.520571	353.376405	1.0463E-32
x_2	5	6841.88145	4959.15784	6.9671E-64
x_1 x_2	15	311.107671	75.1660712	4.5124E-28
Error	48	13.2446		
Corridas totales	71	7458.75429		

x_1 :Tipo de floculante; x_2 :pH; x_1x_2 : Interacción (Tipo de floculante-pH); GL : Grados de Libertad; SC : Suma de cuadrados; F : Valor de F; *p* : Valor de probabilidad.

Esto indica que al menos un tipo de floculante, un valor de pH, y una interacción de pH tuvieron un efecto significativo ($p < 0.05$) en el desempeño de los floculantes en terminos de reducción de la turbidez (NTU). Por lo que, el análisis de varianza se realizó nuevamente considerando sólo los biofloculantes obtenidos por maceración y microondas del diseño de la matriz experimental, y de esta forma identificar el efecto del método de extracción sobre el desempeño del biofloculante de *A. vera*.

Los resultados de este análisis se muestran en la Tabla 6. Se puede observar que el valor estadístico de F indica que no existe diferencia significativa entre el desempeño del biofloculante obtenido por maceración y el biofloculante obtenido por microondas (0.000184 < 4.2596). Contrario a lo anterior, el pH si muestra un efecto significativo ($p < 0.05$) sobre el desempeño de los biofloculantes independientemente del método de extracción evaluado acorde al valor estadístico de F (2988.6658 > 2.62065).

Tabla 6. Tabla de analisis de varianza ANOVA del efecto del método de extracción y del pH sobre sobre el desempeño de los biofloculantes.

Términos del modelo	GL	SC	F	*p*	Valor crítico F
x_1	1	4.44 E-05	0.000184	0.98928	4.259677
x_2	5	3608.856	2988.666	1.05E-32	2.620654
x_1 x_2	5	0.000189	0.000156	0.99999	2.620654
Error	24	5.796067			
Corridas Totales	35	3614.652			

x_1: Tipo de floculante; x_2: pH; x_1x_2: Interacción (Tipo de floculante-pH); GL: Grados de Libertad; SC: Suma de cuadrados; F: Valor de F; p: Valor de probabilidad.

Tabla 4. Diseño de la matriz experimental y resultados obtenidos de la variable de respuesta en el desempeño de los floculantes.

No. de corrida experimental			Factores		Variables de Respuesta		
			x_1	x_2	% Reducción NTU		
1	2	3	-2	-3	66.34	66.15	65.98
4	5	6	-2	-2	50.46	50.38	49.99
7	8	9	-2	-1	52.78	51.88	52.68
10	11	12	-2	1	75.36	75.36	75.01
13	14	15	-2	2	76.75	76.34	76.05
16	17	18	-2	3	59.15	58.56	58.58
19	20	21	-1	-3	76.87	76.78	75.35
22	23	24	-1	-2	56.86	56.64	55.46
25	26	27	-1	-1	52.78	51.88	52.68
28	29	30	-1	1	72.41	71.98	72.9
31	32	33	-1	2	77.76	77.79	76.07
34	35	36	-1	3	69.58	69.88	68.68
37	38	39	1	-3	77.64	77.64	77.31
40	41	42	1	-2	55.85	55.77	54.65
43	44	45	1	-1	53.86	53.83	53.58
46	47	48	1	1	74.74	74.73	74.13
49	50	51	1	2	78.16	78.2	78.58
52	53	54	1	3	68.52	69.73	68.04
55	56	57	2	-3	77.31	77.63	77.66
58	59	60	2	-2	55.02	55.04	56.19
61	62	63	2	-1	53.86	53.84	53.58
64	65	66	2	1	74.31	74.35	74.94
67	68	69	2	2	78.19	78.15	78.58
70	71	72	2	3	69.69	68.62	67.96

Por otro lado, al interpretar el efecto de la interacción (biofloculante por maceración-pH y biofloculante por microondas-pH), los resultados del análisis de varianza indican que el efecto de interacción no sobrepaso a un efecto principal. En este caso, el valor de F no significativo para la interacción nos indica que no hay un efecto de interacción entre las variables (0.000156428 < 2.620654148). En otras palabras, no existe una diferencia significativa entre los rangos de pH evaluados relacionada al biofloculante de *A. vera* obtenido por maceración y al obtenido por microondas.

Esto confirma que el desempeño de los biofloculantes obtenidos en el presente trabajo a partir de *A. vera* no sólo compiten con floculantes comerciales como el sulfato de aluminio ($Al_2(SO_4)_3$), sino que, es más eficiente y estable en forma de polvo en comparación con el gel; minimizando el riesgo de modificación en las propiedades reológicas y la capacidad de floculación por descomposición de los polisacáridos durante el almacenamiento y manejo del gel en fresco; así como de contaminación microbiana (Sonawane et al., 2020). Complementariamente a estos resultados, se ha encontrado que el gel de *A. vera* permite la remoción de arsénico V con valores de remoción similares al de otros floculantes naturales como el quitosano y superiores al de gomas como la xantana; también obteniendo niveles de concentración aceptables para el saneamiento de agua (Venegas-García et al., 2024) confirmándolo como una alternativa sostenible de floculante natural en sistemas de tratamiento de agua.

CONCLUSIÓN

Los métodos de extracción evaluados no tuvieron impacto sobre las propiedades fisicoquímicas, estructurales, reológicas y floculantes del biofloculante. El uso de los modelos reológicos de Newton y de Otswald o Ley de la potencia indicó un comportamiento no Newtoniano, específicamente del tipo pseudoplástico, tanto en el gel como en el biofloculante obtenido (R^2 >0.950 n >1). Los resultados de remoción de turbidez confirmaron que el desempeño del biofloculante obtenido compite con floculantes comerciales como el sulfato de aluminio. Además, que su uso es más eficiente y estable en su forma de polvo en comparación con el gel de *A. vera* (fresco); minimizando el riesgo de modificación en las propiedades reológicas y la capacidad de floculación por descomposición de los polisacáridos durante el almacenamiento y manejo del gel en fresco; así como de la contaminación microbiana. La aplicación de la sábila (*A. vera*) del municipio de Tierra Blanca, Veracruz representa una alternativa sostenible, ecologica y amigable con el ambiente, para sustituir el uso de floculantes sintéticos en sistemas de tratamiento de agua. Adicionalmente, la extracción por microondas ha demostrado ser un proceso que permite minimizar el tiempo de extracción, el uso de solventes y obtener biofloculantes con características fisicoquímicas, estructurales, reológicas y capacidad de floculación similares a los obtenidos por métodos convencionales.

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Increased Antioxidant Activity of *Flourensia cernua* by Fermentation with Kombucha

Incremento de la actividad antioxidante de *Flourensia cernua* mediante Fermentación con Kombucha

Rodríguez-González, J.G.[3], Ascacio Valdés, J.A.[2], Meléndez Rentería, N.P.[4], Ríos-Saldaña, C.A.[3], Rodríguez-Martínez, L.M.[3], Laredo Alcalá, E.I.[1], De León-Zapata, M.A.[1*]

[1]*Centro de Investigación para la Conservación de la Biodiversidad y Ecología de Coahuila, Universidad Autónoma de Coahuila, 27540, Cuatro Ciénegas, Coahuila, México.*
[2]*Departamento de Investigación en Alimentos, Facultad de Ciencias Químicas, Universidad Autónoma de Coahuila, 25280, Saltillo, Coahuila, México.*
[3]*Centro de Estudios e Investigaciones Interdisciplinarios, Universidad Autónoma de Coahuila, 25350, Saltillo, Coahuila, México.*
[4]*Centro de Investigación e Innovación Científica y Tecnológica, Universidad Autónoma de Coahuila, 25070, Saltillo, Coahuila, México.*

**Corresponding Author:* miguel.leon@uadec.edu.mx

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Resumen
Flourensia cernua es conocida como hojasén y se utiliza para tratar problemas gastrointestinales por su contenido de compuestos fenólicos. Estos compuestos son estructuras químicas antioxidantes que se encuentran polimerizadas en tejidos vegetales, por lo que es necesaria su hidrólisis mediante bioprocesos sustentables como la fermentación. El kombucha es un cultivo simbiótico de varias bacterias y levaduras, capaces de producir enzimas para la liberación de los taninos. El presente estudio se enfocó en incrementar la actividad antioxidante de *F. cernua* mediante un proceso de fermentación de kombucha durante 2 semanas a temperatura ambiente. Por lo que se obtuvieron infusiones acuosas fermentadas y no fermentadas (control) para medir su composición química y antioxidante. La infusión de *F. cernua* fermentada con kombucha aumentó significativamente el contenido de taninos totales y el potencial antioxidante. La actividad antioxidante se atribuye principalmente a flavononas, lignanos, ácidos hidroxicinámicos y flavonoides, identificados mediante HPLC-MS en infusiones fermentadas de hojas de *F. cernua*. Los resultados obtenidos sugirieron que la fermentación es un bioproceso prometedor, simple y seguro que podría mejorar las propiedades biológicas de las plantas comestibles menos utilizadas como el hojasén *F. cernua*.

Palabras clave: Antioxidante, bioproceso, Cuatro Ciénegas, *Flourensia cernua*, sustentabilidad.

Abstract
Flourensia cernua, known as tarbush, treats gastrointestinal problems due to its phenolic compound content. These compounds are antioxidant chemical structures found polymerized in plant tissues, requiring their hydrolysis through sustainable bioprocesses such as fermentation. Kombucha is a symbiotic culture of several bacteria and yeasts, which can produce enzymes to release tannins. The present study focused on increasing the antioxidant activity of *F. cernua* through a kombucha fermentation process for 2 weeks at room temperature. Obtained fermented and non-fermented aqueous infusions (control) to measure their chemical and antioxidant composition. Infusion of *F. cernua* fermented with kombucha significantly increased the total tannin content and antioxidant potential. Antioxidant activity attributed to flavanones, lignans, hydroxycinnamic acids, and flavonoids identified by HPLC-MS in fermented infusions of *F. cernua*. The results suggested that fermentation is a promising, safe, and straightforward bioprocess that could improve the biological properties of less-used edible plants such as *F. cernua*.

Keywords: Antioxidant, bioprocess, Cuatro Ciénegas, *Flourensia cernua*, sustainability.

INTRODUCTION

Flourensia cernua is a perennial shrub that grows in semiarid areas. Found in the deserts of Chihuahua and Sonora, where it has commonly been used in traditional medicine for the treatment of stomach pain, diarrhea, dysentery, purgative (Jasso de Rodríguez et al., 2019), antirheumatic, venereal diseases, herpes, bronchitis, chickenpox, and common cold (Ventura et al., 2009).

In addition, leaf extracts of *F. cernua* reported to have antioxidant and antifungal (Jasso de Rodríguez et al., 2011; De León-Zapata et al., 2013; De León-Zapata et al., 2016; Jasso de Rodríguez et al., 2017), insecticidal (Téllez et al., 2001), antibacterial (Méndez et al., 2012) and antitumor (MacRae & Towers, 1984) properties.

The biological activity of *F. cernua* is due to its chemical composition mainly by phenolic compounds such as long-chain hydrocarbons, lactones (Jasso De Rodríguez et al., 2007), saponins (Méndez et al., 2012), terpenes (Estell et al., 2013), condensed tannins, (De León Zapata et al., 2013) and flavonoids (Álvarez-Pérez et al., 2020).

Currently, for the extraction of phenolic compounds from *F. cernua*, solvents such as water, methanol, ethanol, hexane, chloroform and diethyl ether have been used; in addition to a solid fermentation process of *F. cernua* leaves with filamentous fungi (Guerrero et al., 2007; Jasso-De Rodríguez et al., 2007; De León Zapata et al., 2013; De León Zapata et al., 2016). However, *F. cernua* is a non-timber forest resource with great biotechnological potential that is poverly exploited in northern Mexico's arid zones.

On the other hand, kombucha is a beverage obtained by fermentation with a symbiotic culture of several indigenous bacteria (*Acetobacter* and *Gluconobacter*) and yeasts (*Saccharomyces* spp and non-*Saccharomyces* spp) (Malbasa et al., 2015). Most studies suggest that "kombucha" comes from Southeast Asia, Japan, Tibet, or Manchuria and dates back thousands of years (Jarrell et al., 2000). An undulating cellulose film and an acidic liquid broth are the two portions that form "kombucha." Furthermore, kombucha drinks have been claimed to be a prophylactic agent beneficial to health (Villarreal-Soto et al., 2018).

Nowadays, kombucha preparation is not limited to sweetened black tea. Substrates, such as fruit drinks, wine, milk, herbal teas, lemon balm, and green tea, can be used instead of tea. Some new substrates stimulate kombucha fermentation better than the original kombucha tea (Vitas et al., 2013).

Therefore, in the present study, *F. cernua* plant material was subjected to liquid fermentation with kombucha to increase its antioxidant properties.

MATERIALS AND METHODS

Material and reagents

The leaves of *F. cernua* were collected in the rural community of La Vega, Cuatro Ciénegas, Coahuila, Mexico, belonging to Ramsar site number 734 of Cuatro Ciénegas, recognized for its high biodiversity and endemism. The plant material was dehydrated for 10 days, pulverized, and stored in plastic bags at room temperature (25 °C) until use. Moreover, the reagents that correspond to 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Folin Ciocalteu reagent, gallic acid, ethanol, acetonitrile, acetic acid, sodium carbonate, HCl, $K_2S_2O_8$, KH_2PO_4, MeOH, NaOH y Na_2HPO_4, were purchased from Sigma Aldrich.

Obtaining the kombucha culture and inoculum preparation

Traditional kombucha was cultivated in the laboratory of the Food Research Department of the Faculty of Chemical Sciences of the Autonomous University of Coahuila (Mexico) and used as the initial starter for fermentation. The inoculum was according to the methodology described by Jayabalan et al. (2014), with adaptations. Dried leaves of *F. cernua* were used as raw material for the infusion. Sucrose (50 g) was dissolved in 1000 mL of purified water and heated to 90 °C. Then, 12 g of leaf powder was introduced into a small muslin cloth bag and placed in boiling water for 5 minutes. Afterward, the preparation was allowed to cool at 30 °C before adding 7 g of the kombucha strain. The kombucha strain was cultured in the previously cooled medium for 14 days at a temperature of 25 °C.

To prepare the unfermented infusion as a control, 12 g of leaf powder was introduced into a small muslin cloth bag, which was placed in 1000 mL of purified water at 90 °C for 5 minutes. Afterward, the aqueous infusion was allowed to cool to 30 °C, stored in a previously disinfected amber plastic container, and frozen at -5 °C until use.

Determination of pH and TSS

The total soluble solids (TSS) content of the fermented and unfermented samples was determined by refractometry using a digital refractometer (Atago Co., Tokyo, Japan) with automatic temperature compensation. The results are in % (w/w sucrose concentration). The pH values of the fermented and unfermented samples were measured using an electronic pH meter (pH2700, Eutech, Thermo Fisher Scientific, USA).

Total yeast count

Fermented samples were taken at 3, 7, 10, and 14 days of fermentation. After gently mixing the fermented brew, 1 mL samples were placed in Eppendorf tubes. A Thoma cell counting chamber (Thomas Scientific, Swedesboro, NJ, USA) was used to count yeast. For microscopic observations, one drop of fermented brew was placed into a coverslip and observed on the stage of a binocular microscope (Laborlux 12 microscope, Leitz, Midland, ONT, Canada) for further observation at 10 or 40x magnification.

Determination of antioxidant activity

DPPH assay performed as described by De León-Zapata et al. (2021). The cation radical ABTS was synthesized by a 7 mM ABTS solution reaction with a 2.45 mM $K_2S_2O_8$ solution. The mixture was kept at 23 ± 1 ºC in the dark for 16 h. Afterward, the ABTS solution was diluted with ethanol until a UV- Vis spectrophotometer achieved an absorbance of 0.7 at 734 nm. 10 µL of the sample (fermented and non-fermented infusion) was added in the reaction cuvette immediately after 1 mL of ABTS solution was added. After 10 min, the percentage inhibition of absorbance at 734 nm was calculated for each concentration relative to the blank absorbance (ethanol).

The DPPH radical is characterized by an unpaired electron, a free radical stabilized by resonance. A solution of DPPH radical at a concentration of 60 mM by diluting with methanol was prepared. 100 µL of the sample (fermented and non-fermented infusion) was added in test tubes covered with foil, plus 2.9 mL of DPPH solution, and allowed to stand for 30 min. The absorbance was recorded at a wavelength of 517 nm.

The results were expressed in DPPH and ABTS radicals percentage of inhibition (%).

Total tannins

Total tannins were determined according to the methodology described by Wong-Paz et al. (2014) using the Folin-Ciocalteu reagent. In a microplate well, 20 µL of sample was added. Subsequently, 20 µL of Folin-Ciocalteu's reagent was added and mixed for 5 min. Subsequently, 20 µL of sodium carbonate (10 mM) was added to the mixture and allowed to react for another 5 min. Finally, 125 µL of distilled water was added, and the absorbance was read at 790 nm using a microplate reader. The results were expressed as milligrams of gallic acid equivalents (GAE) per milliliter of fermented and non-fermented infusion (mg GAE/mL) according to a gallic acid standard curve (0-1,000 mg/L; R^2 = 0.997).

High-performance liquid chromatography-mass spectrometry assay (HPLC-MS)

Samples of fermented and non-fermented infusions were filtered through a nylon membrane (pore size 0.45 µm), 1.5 mL of infusion was placed in 1.8 mL capacity vials for HPLC analysis (Alliance HPLC, Water e2695) with a UV-vis photodiode array detector at 280 nm. The determination was performed under the following operating conditions: Denali C18 column, mobile phase A was ethanol (wash phase), phase B was acetonitrile, and phase C was 3 % acetic acid in gradient elution, and the injection volume was 10 µl. A Varian 500/MS mass spectrometry with a flow rate of 1 mL/minute was used, and the detection of the mass range was 100-2000 for 10 minutes (Ascacio-Valdés et al., 2010).

Statistical analysis

Results were evaluated using ANOVA with six replicates. Values reported are the average of measurements and were compared using the Tukey multiple range test with a significance level of $p < 0.05$. Data analysis was conducted using the Statistica 7.0. Pearson's correlation was calculated using Microsoft Excel.

RESULTS AND DISCUSSIONS

Total yeast count and determination of pH and TSS

The results from the total yeast count indicated that after 10 days of incubation, the total yeast count increased slightly from 1.6E+07 to 1.7E+07 cells/mL in the liquid (Table 1). At the end of fermentation, yeast growth remained constant at 1.7E+07 cells/mL (Table 1).

Table 1. Effect of the fermentation of infusions of *F. cernua* with kombucha on yeast growth, pH, and total soluble solids (TSS).

Fermentation time (Days)	Yeast count (log CFU/mL)	pH	TSS (%)
0	0^{e}	7.0^{e}	5.4^{e}
3	$1.00E+06^{d}$	6.3^{d}	5.1^{d}
7	$1.30E+07^{c}$	5.7^{c}	4.9^{c}
10	$1.60E+07^{b}$	5.4^{b}	4.4^{b}
14	$1.70E+07^{a}$	5.0^{a}	4.1^{a}

Within each column, different letters represent a significant difference ($P < 0.05$). CFU: Colony Forming Units.

The pH values of the fermented *F. cernua* leaf infusion had a significant decrease ($p \leq 0.05$) compared to the non-fermented control (pH = 7). A rapid decrease in the pH of the infusion was observed during the first week, falling to pH = 5 at the end of fermentation (Table 1).

Furthermore, the total soluble solids (TSS) values decreased significantly ($p \leq 0.05$) from 5.4% to 4.1% as the fermentation process proceeded (Table 1). The role of yeasts in kombucha fermentation is important because they are known to be responsible for ethanol production through the hydrolysis of sucrose (Watawana et al., 2015; Sievers et al., 1995). The behavior observed in the present study, where sucrose was consumed by kombucha yeasts (Table 1). Moreover, acetic acid bacteria use ethanol to produce acetic acid, the main fermentation product of kombucha and the main reason for decreased pH (Jayabalan et al., 2014) (Table 1). The results were consistent with previous work reporting acetic acid and ethanol content changes during kombucha fermentation (Abbott & Ingledew, 2004; Sievers et al., 1995; Yang et al., 2016). The pH changes obtained are similar to those found by Velicanski et al. (2013), who used *Lamiaceae* as a substrate for kombucha fermentation in small bioreactors. Several investigations have reported that low pH has many beneficial effects, such as protecting the bioactivity of phenolic compounds and the safety of fermented infusions against pathogenic microorganisms (Lucera et al., 2012).

Antioxidant activity and total tannins

Figures 1 and 2 show the antioxidant activity values measured as the capacity of the infusion to reduce DPPH and ABTS free radicals, respectively.

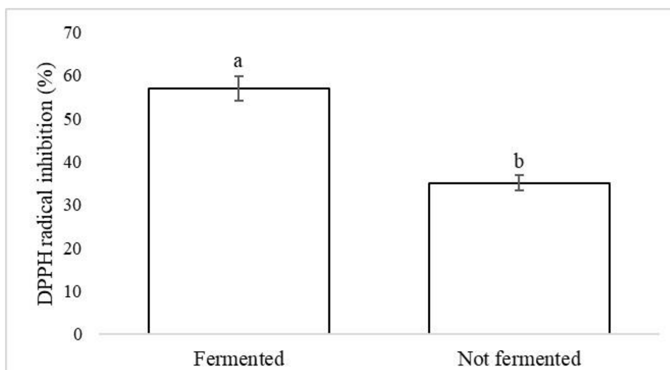

Figure 1. Antioxidant activity of fermented and unfermented *F. cernua* infusions by DPPH assay. Different letters indicate significant differences at $p < 0.05$.

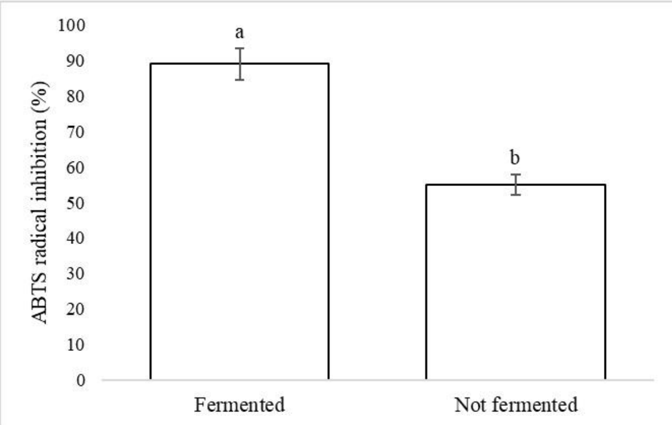

Figure 2. Antioxidant activity of fermented and unfermented *F. cernua* infusions by ABTS assay. Different letters indicate significant differences at $p < 0.05$.

There was a significant increase ($p \leq 0.05$) in the antioxidant activity by DPPH (Figure 1) and ABTS (Figure 2) of the fermented samples compared to the non-fermented ones. The fermented samples showed a higher antioxidant power for scavenging ABTS radicals (Figure 2) than the DPPH radical (Figure 1). The obtained values of antioxidant activity of the fermented samples by ABTS and DPPH were 89% and 57%, respectively, compared to the non-fermented samples (33%).

Figure 3 shows a significant increase ($p \leq 0.05$) can be observed in the total tannin content of the fermented samples (1152 mg gallic acid equivalents/mL) compared to the unfermented samples (109 mg of gallic acid equivalents/mL). The total tannin content of the unfermented samples (109 mg gallic acid equivalents/mL) (Figure 3) was higher than that reported by Méndez et al. (2012) in an aqueous extract of *F. cernua* (4.76 of gallic acid equivalents/g) because the extract was subjected to 60°C for a longer time (7 hours) than in the present study (10 minutes at 90°C), which may accelerate the degradation of phenolic compounds.

The high values of total tannins in the fermented samples (Figure 3) are much higher than those reported by other authors in extracts of *F. cernua* using water, ethanol, hexane and ether, methanol-chloroform as extraction solvents (De León-Zapata et al., 2016; Álvarez-Pérez et al., 2020; Guerrero et al., 2007).

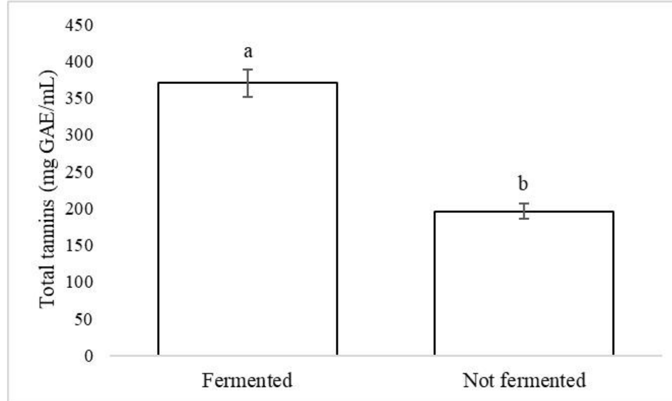

Figure 3. The total tannin content of fermented and unfermented *F. cernua* infusions. Different letters indicate significant differences at $p < 0.05$.

This behavior relates to the biotransformation of secondary metabolites present in *F. cernua* by yeasts, bacteria, and fungi in kombucha (Watawana et al., 2015). Some studies have corroborated this behavior by demonstrating that fermentation is a bioprocess that allows obtaining tannin-rich beverages from black tea, sweetened lemon balm, and different fruits and vegetables (Velicanski et al., 2013; Yang et al., 2016). Fermented samples of *F. cernua* showed a higher antioxidant power for scavenging ABTS radicals (Figure 2) than DPPH radicals (Figure 1); this is due to the sensitivity of the ABTS radical as it is a structure that easily reacts with hydrophilic and lipophilic compounds (Jasso de Rodríguez et al., 2023), and reducing agents (De León-Zapata et al., 2016) such as total and reducing sugars present in tarbush (Belmares et al., 2009). On the other hand, the DPPH radical reacts with hydrophilic compounds such as gallic acid (Álvarez et al., 2020). The antioxidant activity is higher than that reported in an aqueous extract of fermented tarbush in a solid medium (De León-Zapata et al., 2013). The amount of total tannins and the radical scavenging activity of DPPH and ABTS showed a concentration-dependent relationship. This is consistent with the fact that the antioxidant activity of phenolic compounds is mainly due to the number of hydroxyl groups, as well as their redox properties (De León-Zapata et al., 2013).

Tannins are highly soluble due to the interaction of water with hydroxyl groups and carboxylic acids (Méndez et al., 2012). A high content of phenolic compounds could initially suggest a good antioxidant capacity (Cheung et al., 2003). The results show that at the highest concentration of total tannins (Figure 3) in the fermented samples, the highest values of antioxidant activity by DPPH (Figure 1) and ABTS (Figure 2) were obtained due to the greater amount of available hydroxyl groups. Flavonoids and hydrolyzable tannins contain in their chemical structure a variable number of hydroxyl groups (Jasso de Rodríguez et al., 2023), which are involved in the neutralization of free radicals by electron donation and, therefore, influence the antioxidant activity (De León-Zapata et al., 2013).

High-performance liquid chromatography-mass spectrometry assay (HPLC-MS)

Figure 4 shows the chromatographic profile of the main phenolic compounds of unfermented *F. cernua* samples at different retention times where the main signals correspond to (A) Caffeic acid 4-O-glucoside (Hydroxycinnamic acids), (B) Syringaresinol (Lignans), (C) Phloretin (Dihydrochalcones), (D) Caffeoyl tartaric acid (Hydroxycinnamic acids) and (E) Apigenin galactoside-arabinoside (Flavonoid).

Furthermore, Figure 5 shows the chromatographic profile of the main phenolic compounds of the fermented samples of *F. cernua* at different retention times where the main signals correspond to (A) Pinocembrin (Flavanones), (B) Syringaresinol (Lignans), (C) 4-Caffeoylquinic acid (Hydroxycinnamic acids), (D) Caffeoyl tartaric acid (Hydroxycinnamic acids) and (E) Apigenin galactoside-arabinoside (Flavonoid), not yet reported in extracts of *F. cernua*.

These results are similar to those reported by De León-Zapata et al. (2016) and Álvarez-Pérez et al. (2020), who reported the identification of flavonoids such as luteolin 7-O-rutinoside, 6-C-glucosyl-8-C-arabinosyl apigenin and apigenin galactoside arabinoside in resins obtained from an aqueous extract of *F. cernua*, and by Aranda-Ledesma et al. (2022), who identified apigenin-6-C-glucosyl-8-C-arabinoside by UPLC/QToF-MS2 in *F. cernua* essential oil.

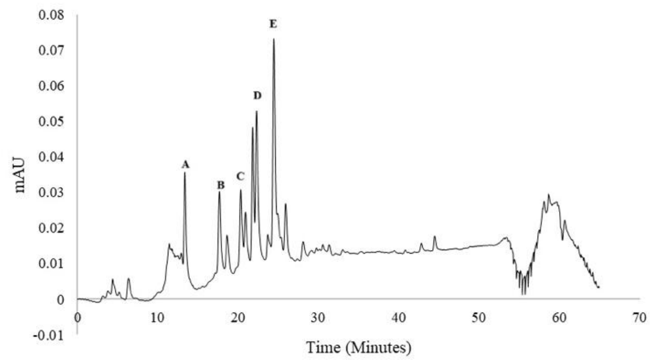

Figure 4. Chromatographic profile of the main phenolic compounds recorded in unfermented samples of *F. cernua* at different retention times. (A) Caffeic acid 4-O-glucoside, (B) Syringaresinol, (C) Phloretin, (D) Caffeoyl tartaric acid y (E) Apigenin galactoside-arabinoside.

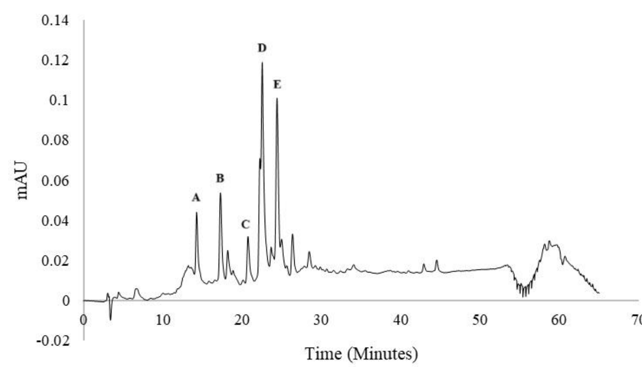

Figure 5. Chromatographic profile of the main phenolic compounds recorded in fermented samples of *F. cernua* at different retention times. (A) Pinocembrin, (B) Syringaresinol, (C) 4-Caffeoylquinic acid, (D) Caffeoyl tartaric acid y (E) Apigenin galactoside-arabinoside.

The presence of a higher amount of phenolic compounds, as in the fermented samples of *F. cernua* (Figure 5), suggests a higher amount of tannins and, therefore, a higher amount of hydroxyl groups (Jasso-De Rodríguez et al., 2007), which are responsible for the antioxidant activity by neutralizing free radicals. These natural phenolic compounds are flavonoids found in foods of plant origin (Scalbert & Williamson, 2000). Tarbush *F. cernua* plant uses these natural chemical compounds as resistance against microorganisms, rodents, insects, etc. (Belmares et al., 2009; Méndez et al., 2012). Fermented infusions of *F. cernua* represent a rich natural source of phenolic compounds with antioxidant activity; this is more so when compounds are extracted from natural and organic hydrolytic bioprocesses such as fermentation with kombucha (Rahmani et al., 2019).

CONCLUSIONS

Phytochemical analysis of fermented and unfermented *F. cernua* infusions showed that fermentation increased the total tannin content and enhanced antioxidant activity. The present results are the first report to identify flavanones, lignans, hydroxycinnamic acids, and flavonoids by HPLC-MS of fermented *F. cernua* leaves infusions obtained by a bioprocess of fermentation with kombucha. *F. cernua* leaf resulted in a promising natural source for the recovery of high-added value compounds with antioxidant activity and, at the same time, increased the added value of this plant widely distributed in semiarid regions of Mexico. Its controlled use only involves harvesting or pruning the upper third of the foliage and would adhere to the Forestry Law on non-timber forest resources. The fermented *F. cernua* leaves infusion with kombucha represents a natural alternative with antioxidant properties, which gives it great therapeutic potential against oxidative processes at the cellular level for the treatment of chronic and degenerative diseases, such as cancer, arthritis, neurodegenerative diseases, and cardiovascular diseases.

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Bioactive Compounds and Biological Properties of *Flourensia cernua*: A Review

Compuestos Bioactivos y Propiedades Biológicas de *Flourensia cernua*: Una Revisión

Rodríguez-Martínez, L.M.[3], Rodríguez-González, J.G.[3], Barrera Martínez, C.L.[1], Ríos-Saldaña, C.A.[3], Laredo Alcalá E.I.[1], Ascacio Valdés J.A.[2], Meléndez Rentería N.P.[4], De León-Zapata M.A.[1*]

[1]*Centro de Investigación para la Conservación de la Biodiversidad y Ecología de Coahuila, Universidad Autónoma de Coahuila, 27540, Cuatro Ciénegas, Coahuila, México.*
[2]*Departamento de Investigación en Alimentos, Facultad de Ciencias Químicas, Universidad Autónoma de Coahuila, 25280, Saltillo, Coahuila, México.*
[3]*Centro de Estudios e Investigaciones Interdisciplinarios, Universidad Autónoma de Coahuila, 25350, Saltillo, Coahuila, México.*
[4]*Centro de Investigación e Innovación Científica y Tecnológica, Universidad Autónoma de Coahuila, 25070, Saltillo, Coahuila, México.*
**Autor para correspondencia: miguel.leon@uadec.edu.mx*

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Resumen

El hojasén *(Flourensia cernua*) es un arbusto perenne del Desierto Chihuahuense en Norteamérica. Las hojas de la planta son muy resinosas, con un olor alquitranado y un sabor amargo y astringente. Los compuestos bioactivos de la resina funcionan como defensas bioquímicas para repeler microorganismos dañinos y animales herbívoros, ya que no se conocen plagas, enfermedades ni animales que ataquen a esta planta. Algunos estudios han demostrado que los extractos de hojasén tienen acción antifúngica en condiciones in vitro contra al menos 10 hongos y 2 bacterias fitopatógenas de importancia económica. Los extractos también han mostrado actividad citotóxica contra líneas celulares de cáncer de mama, así como actividad antiinflamatoria, antiproliferativa y antioxidante. También se ha demostrado el efecto insecticida o repelente contra termitas, *Sitophilus oryzae, Phthorimaea operculella* y *Brevicoryne brassicae*. Por otra parte, pruebas in vitro con microorganismos que atacan al ser humano han indicado que al menos 4 bacterias son susceptibles a los extractos de *F. cernua*. Con base en esta información, es claro el potencial de este arbusto resinoso, propio de las zonas áridas del norte de México, para la obtención sustentable de productos de alto valor agregado y con menor impacto ambiental.

Palabras clave: Efectividad biológica, Extractos naturales, Metabolitos secundarios, Sustentabilidad, Hojasén.

Abstract

Tarbush *Flourensia cernua* is a perennial shrub from the Chihuahuan Desert of North America. The plant leaves are very resinous with a tarry odor and a bitter, astringent taste. The bioactive compounds of the resin function as biochemical defenses to repel harmful microorganisms and herbivorous animals, as there are no known pests, diseases, or animals that attack this plant. Some studies have shown that tarbush extracts have antifungal action under in vitro conditions in at least 10 fungi and two phytopathogenic bacteria of economic importance. The extracts also have shown cytotoxic activity against breast cancer cell lines and anti-inflammatory, antiproliferative, and antioxidant activity. The insecticidal or repellent effect against termites *Sitophilus oryzae, Phthorimaea operculella,* and *Brevicoryne brassicae* has also been demonstrated. On the other hand, in vitro tests with microorganisms that attack humans have indicated that at least four bacteria are susceptible to extracts of *F. cernua*. Based on this information, the potential of this resinous shrub from arid areas of northern Mexico for the sustainable production of high-value products with less environmental impact is clear.

Keywords: Biological effectiveness, Natural extracts, Secondary metabolites, Sustainability, Tarbush.

INTRODUCTION

Arid zones represent great potential, where flora and fauna are the product of thousands of years of physiological adaptation for survival. Mexico has a significant and extensive plant biodiversity. Mexico has a wide variety of plants; it is the fourth most prosperous country in the world in this regard (Jasso-De Rodríguez et al., 2011). In parts of northern Mexico, where the climate is semiarid, many wild plants grow in this extreme condition (Jasso-Rodríguez et al., 2012).

A typical case of these conditions is represented by the tarbush *Flourensia cernua* of the Asteraceae family, which is a perennial shrub that grows in semiarid areas and is found in the Chihuahua and Sonoran deserts, covering different Mexican states such as Coahuila, Chihuahua, Durango, Nuevo León, San Luis Potosí, Sonora and Zacatecas (Rodríguez et al., 2006). Different names commonly know Tarbush since it is found both in the United States of America and in Mexico. The names given in the United States of America are tarbush, hojase, American-tarbush, black-brush, varnish-brush, and hojasen. In Mexico, it is known as hojasen, black brush, and tarbush due to its peculiar aroma (Innes & Robin, 2010).

This plant is commonly used as an infusion for the treatment of various gastrointestinal diseases such as stomach pain, diarrhea, and dysentery, and it is used as a purgative (Jasso de Rodríguez et al., 2019), expectorant, and antirheumatic. In addition to these effects, tarbush *F. cernua* extracts have been reported to have antioxidant and antifungal (Jasso-De Rodríguez et al., 2011; Mata et al., 2003; De León-Zapata et al., 2013; De León-Zapata et al., 2016; Jasso de Rodríguez et al., 2017), insecticidal (Towers et al., 1975; Téllez et al., 2001; Molina-Salinas et al., 2006), antibacterial (Molina-Salinas et al., 2006; Méndez et al., 2012) and antitumor (MacRae & Towers, 1984) properties.

The biological activity of tarbush *F. cernua* is due to its chemical composition mainly by bioactive compounds such as long-chain hydrocarbons, lactones, saponins, terpenes, condensed tannins and flavonoids (De León-Zapata et al., 2013; Méndez et al., 2012; Jasso De Rodríguez et al., 2007; Estell et al., 2013; Álvarez-Pérez et al., 2020). For the extraction of active phytochemical compounds, the most commonly used solvents are methanol, ethanol, hexane, chloroform, and diethyl ether (Jasso De Rodríguez et al., 2007; Guerrero Rodríguez et al., 2007).

Therefore, this article aims to carry out an updated review of *F. cernua* in terms of the identified bioactive compounds, toxicity, antioxidant, cytotoxic, insecticidal, antifungal, and antibacterial properties.

Botanical description

F. cernua is a perennial shrub that grows from a network of roots that can spread horizontally across four meters (over 13 ft). Most are shallow, but some extend to five meters (over 16 ft) deep into the soil. It typically grows to a maximum height of about one meter (40 in) but can be as tall as two meters (7 ft). It can grow erect or spreading in shape. It has many branches branching off from the base of the stem (Figure 1).

Figure 1. Branched shrub of *Flourensia cernua*.

The branches are covered with thick, alternately arranged, oval leaves up to 2.5 centimeters (0.98 in) in length, sometimes reaching 4 centimeters (1.6 in). The leaf edges are smooth or wavy (Figure 2).

Figure 2. Thick oval and elongated leaves of *Flourensia cernua*.

The pendulous flower heads contain several yellow disc florets and no ray florets (Innes & Robin, 2010). The fruit is a hairy achene up to 1 centimeter (0.39 in) long. Most parts of the plant are very resinous and have a tarry or hoppy odor and a bitter taste (Mata et al., 2003). Leaf production of *F. cernua* is affected by humidity levels, and it has been observed that the plant first produces small leaves and, during spring, produces larger leaves as humidity increases. Growth occurs early in the year when rainfall is abundant. Flowering occurs in the autumn. The plant generally produces few flowers in dry years. The root network is shallow and vast, with some profound roots, helping it collect water from a wide soil area, another adaptation to its dry habitat (Innes & Robin, 2010).

Bioactive compounds, antioxidant, and antiproliferative properties, and toxicity

The main bioactive compounds of tarbush *F. cernua* reported in the literature are methyl or selenate, ermanine, flourensadiol, dehydrofluorenic acid, long-chain hydrocarbons from tetracosane-4-olide to triacontane-4-olide and lactones, as well as saponins, terpenes (Méndez et al., 2012; Jasso De Rodríguez et al., 2007; Estell et al., 2013), condensed tannins equivalent to catechins (De León-Zapata et al., 2013; Méndez et al., 2012; Belmares et al., 2009; Castillo et al., 2010) and flavonoids such as luteolin 7-O-rutinoside, 6-C-glucosyl-8-C-arabinosyl apigenin and apigenin galactoside arabinoside (De León-Zapata et al., 2016; Álvarez-Pérez et al., 2020). The distribution of volatile bioactive compounds in *F. cernua* leaves of immature, intermediate, and mature ages was also examined (Estell et al., 2013), and the identification of apigenin-6-C-glucosyl-8-C-arabinoside by UPLC/QToF-MS2 in *F. cernua* essential oil (Aranda-Ledesma et al., 2022). Volatile compound extraction was done with ethanol and analyzed with gas chromatography-mass spectrometry. The results demonstrated that the age of *F. cernua* leaves affects the concentration of terpenes (mainly sesquiterpenes) and sampling variability, where 63 compounds differed between leaf age categories. Immature leaves contained higher concentrations of 46 chemicals than intermediate or mature age categories, but intermediate and mature leaves only differed in seven compounds.

It has been shown that the higher the concentration of bioactive compounds in *F. cernua* leaves, the greater the capacity to inhibit free radicals; therefore, there is greater antioxidant activity (De León-Zapata et al., 2016). The yield of bioactive compounds from *F. cernua* leaves is affected by the harvest season, climatic conditions, phenological stage of the plant, plant tissue (leaves, stems, roots, etc.) (Hyder et al., 2005) and solvent polarity (Weng Kong et al., 2012). Tannins are highly soluble due to the interaction of water with hydroxyl groups and carboxylic acids (Méndez et al., 2012). Flavonoids, terpenes, and hydrolyzable tannins contain in their chemical structure a variable number of hydroxyl groups (Martínez-Flores et al., 2002), which are involved in the neutralization of free radicals by electron donation and therefore influence antioxidant activity (De León-Zapata et al., 2013). Bioactive compounds from *F. cernua* have shown greater antioxidant power for scavenging ABTS radicals compared to the DPPH radical (De León-Zapata et al., 2016; Álvarez-Pérez et al., 2020; Jasso de Rodríguez et al., 2023); this is due to the sensitivity of the ABTS radical as it is a structure that easily reacts with hydrophilic and lipophilic compounds (Aranda-Ledesma et al., 2022), and reducing sugars present in *F. cernua* leaves (Belmares et al., 2009). On the other hand, the DPPH radical reacts with hydrophilic compounds such as gallic acid (Álvarez et al., 2008). The antioxidant activity of bioactive compounds from *F. cernua* leaves is higher than that reported in several raspberry varieties rich in ellagitannins (De Ancos et al., 2000). The hepatoprotective effect of *F. cernua* extracts against ischemia-reperfusion-induced damage in Wistar rats was reported due to its potential anti-inflammatory and antioxidant activity (García-Carmona et al., 2024). In 2023, was published the first scientific report on the antiproliferative activity of *F. cernua, Flourensia microphylla*, and *Flourensia retinophylla* extracts on A549 lung cancer cells, and *F. cernua* demonstrated the best anti-inflammatory activity. Therefore, these extracts are natural anti-inflammatory agents to control lung cancer (Jasso de Rodríguez et al., 2023).

Currently, few studies are determining the toxicity of bioactive compounds from *F. cernua* leaves. In 1980, the activity was reported for the first time, the cytotoxic activity of pure benzopyrans and benzofurans from *F. cernua* using red blood cells and measuring the hemoglobin released in cell destruction (Towers et al., 1980). The results showed that benzopyrans were more active than benzofurans. Bioassay-directed fractionation of a CH2Cl2-MeOH (1:1) extract of the aerial parts of *F. cernua* has also been reported, from which three phytotoxic compounds such as dehydrofluorenic acid, fluorensadiol, and methyl orselinate were isolated (Mata et al., 2003). The results indicated that the phytotoxic compounds caused a significant inhibition in the radicle growth of *Amaranthus hypochondriacus* and *Echinochloa crus-galli*, in addition to interacting with bovine brain calmodulin and inhibiting the activation of the calmodulin-dependent cAMP phosphodiesterase enzyme. It has been shown that crude leaf extracts of *F. cernua* and its fractions are cytotoxic against human breast cancer cells (Molina-Salinas et al., 2006).

The shrub *F. cernua* has rapidly increased in prevalence within the Chihuahuan Desert grasslands and is comparable to alfalfa in nutrient content such as protein and fiber. Increasing the amount of *F. cernua* leaves in cattle diets may improve diet quality but reduce their prevalence and cause toxicosis due to their high consumption due to their bioactive compound content, which is why it is recommended to remove them for use as cattle feed (Fredrickson et al., 2007). In one study, the acute toxicity of an ethanolic extract of *F. cernua* leaves in rats using the alternative fixed dose method, where it was found that the LD50 of the extract was above 2000 mg/kg, with signs of mild toxicity and there were no changes in body weight or mortality in the animals (Zavala et al., 2010).

In vitro antifungal properties

The efficacy of bioactive compounds from tarbush *F. cernua* in inhibiting the growth of phytopathogenic fungi that attack fruit crops of commercial interest has been demonstrated (Table 1).

Table 1. Antifungal activity of *F. cernua* leaf extracts rich in bioactive compounds

Extracts	Dose	Phytopathogenic fungi	References
Ethanolic extract of leaves	10 µl/L to 100 µl/L	*Alterniasp.*, *Rhizoctonia solani*, and *Fusarium oxysporum*	(Jasso De Rodríguez et al., 2007)
Methanol: chloroform extract of leaves	4000 mg/L	*Colletotrichum gloesporoides*	(Guerrero Rodríguez et al., 2007)
Hexane leaf extract	4000 mg/L	*Colletotrichum gloesporoides*	(Guerrero Rodríguez et al., 2007)
Ethanolic extract of leaves	2000 mg/L	*Colletotrichum gloesporoides* and *Penicillum digitatum*	(Guerrero Rodríguez et al., 2007)
Leaf extract with lanolin as a solvent	2000 mg/L	*Rhizoctonia solani*	(Castillo et al., 2010)
Leaf extract with cocoa butter as a solvent	1000 mg/L	*Rhizoctonia solani*	(Castillo et al., 2010)
Resin powder from aqueous extract of leaves	1,519 to 3,310 mg/L	*Rhizopus stolonifer, Botrytis cinerea, F. oxysporum, and C. gloesporioides*	(De León-Zapata et al., 2016)
Ethanolic extract of leaves	2163 mg/L	*F. oxysporum and R. stolonifer.*	(Jasso de Rodríguez et al., 2017)
Ethanolic extract of branches	4240 mg/L	*F. oxysporum and R. stolonifer*	(Jasso de Rodríguez et al., 2017)
Ethanolic extract of leaves and branches	1692 mg/L	*R. stolonifer*	(Jasso de Rodríguez et al., 2017)
Resin powder from aqueous extract of leaves	125 to 1000 mg/L	*F. oxysporum, B. cinerea, Penicillium sp., Alternaria alternata, R. stolonifer, Mucor sp., Sclerotinia sclerotorium, and C. gloesporioides*	(Álvarez-Pérez et al., 2020)

Bioactive compounds from *F. cernua* leaf extracts obtained with hexane, ethanol, and a methanol-chloroform mixture have been reported to have a higher inhibitory activity against *C. gloeosporioides* (Guerrero Rodríguez et al., 2007). Moreover, higher inhibition values against *F. oxysporum* have been obtained using ethanol as a solvent. It should be noted that there are few studies evaluating the antifungal power of bioactive compounds from *F. cernua* leaves against *B. cinerea* and *R. stolonifer* (Jasso De Rodríguez et al., 2007). However, it has been reported that tarbush tannins obtained with lanolin and cocoa butter as solvents inhibited 70% of *R. solani* growth at a concentration of 500 mg/L of tannins (Castillo et al., 2010). As well was a study where it was reported that the resin of an aqueous extract of *F. cernua* leaves showed an inhibition of mycelial growth at a minimum inhibitory concentration of 1362 mg/L against phytopathogenic fungi of *F. oxysporum, B. cinerea, Penicillium* sp., *A. alternata, R. stolonifer, Mucor* sp., *S. sclerotorium*, and *C. gloesporioides*, thus representing a viable alternative to counteract the use of chemical compounds in agriculture (Álvarez-Pérez et al., 2020). The great antifungal effectiveness of the bioactive compounds of *F. cernua* leaves is attributable to the phenolic compounds present as hydroxyl groups of hydrolyzable tannins equivalent to gallic acid, flavonoids, and terpenes, among others.

Insecticidal and bactericidal properties

Few reports mention the insecticidal properties of bioactive compounds from tarbush *F. cernua*, among which stands out the study of the insecticidal activity of benzofuran 7-methoxy-2-isopropenyl-5-acetyl-2,3 dihydrobenzofuran-3-ol-cinnamate from tarbush *F. cernua*, which demonstrated its activity as a juvenile hormone causing anatomical malformations, retention of juvenile characteristics and sterility in treated insects from their second to fourth stage of development (Towers et al., 1975). In addition, the termiticidal activity of fractions of bioactive compounds from tarbush *F. cernua* with hexane, diethyl ether, and ethanol has also been reported (Téllez et al., 2001). The evaluation of the insecticidal activity of crude extracts of *F. cernua* leaves extracted with solvents of variable polarity on three insect pests of agricultural importance such as *Sitophilus oryzae*, *Phthorimaea operculella* and *Brevicoryne brassicae*, as well as the repellent or attraction effect on *Sitophilus oryzae* has also been reported (Molina-Salinas et al., 2006). The results showed that the extract's hexane fraction had the most excellent insecticidal effect against *B. brassicae*, with a 100% mortality at a concentration of 10,000 µl L-1 at 24 h. In addition, the hexane fraction showed an insecticidal effect by inciting repellency to *S. oryzae* at 5 and 45 days. It is worth mentioning that the repellency effect caused by the hexane fraction may be due to the volatile substances it contains, such as borneol and camphor. Few works have demonstrated the bactericidal effect of the bioactive compounds of tarbush *F. cernua*, among which the evaluation of the effect of the hexane extract of the aerial parts of leaves in *F. cernua* at a minimum inhibitory concentration (MIC) of 50 and 25 µg L^{-1} to inhibit the growth of the H37Rv and CIBIN strains of *Mycobacterium tuberculosis*: UMF: 15: 99 (Molina-Salinas et al., 2006) as well as the work carried out by Peralta (2006) who evaluated the antibacterial activity of an extract of tarbush *F. cernua* obtained with a mixture of hexane, ether, ethanol and methanol-chloroform, at different doses, in phytopathogenic bacteria such as *Pseudomonas cichorii* and *Xanthomonas axonopodis* pv. *Phaseoli.* The results indicated that the hexane extract of *F. cernua* at a concentration of 4000 µl ml-1 showed the most significant inhibition on both phytopathogenic bacteria. The bactericidal effect of tarbush *F. cernua* extracts obtained by the Soxhlet method using water, ethanol, and alternative organic solvents (lanolin and cocoa butter) has also been demonstrated against pathogenic bacteria that attack humans, such as *Enterobacter aerogenes, Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* (Méndez et al., 2012).

Other relevant applications of bioactive compounds of *F. cernua*

The bioactive compounds of the tarbush *F. cernua* have also been the subject of other types of studies in the medical field, where the microencapsulation of ethanolic extracts of three species of *Flourensia* spp. (*Flourensia cernua, Flourensia microphylla* and *Flourensia retinophylla*) furthermore, the evaluation of the controlled release of the microencapsulated extracts in a gastrointestinal system in vitro has been reported (Jasso de Rodríguez et al., 2019). Encapsulation was carried out using the alginate gelation technique. The results showed that microencapsulation managed to preserve the antioxidant power of the extracts and protect them until they reached the in vitro gastrointestinal system. It is worth mentioning that this work was a pioneer in laying the foundations for applying *Flourensia* spp. extracts in microsystems to take advantage of their benefits for human health.

In the postharvest field, extracts rich in bioactive compounds from tarbush have been used in fruits and vegetables to increase shelf life and prevent microbial contamination during storage. For example, microemulsions with candelilla wax, distilled water, gum arabic, and jojoba oil have been prepared to encapsulate a fermented extract of *F. cernua* tarbush polyphenols to increase shelf life and improve the quality of Golden Delicious apples (De León-Zapata et al., 2015). A nanoemulsion based on candelilla wax, distilled water, gum arabic, glycerol, tween 80, and jojoba oil has also been formulated, with a crude extract of *F. cernua* as an antioxidant and antimicrobial additive. The nanoemulsion was made by high-shear hot stirring and increased the nutritional quality of apples at the laboratory and industrial levels (De León-Zapata et al., 2017; De León-Zapata et al., 2018). The effects of nanolaminate coatings of alginate and chitosan nanolayers incorporated with *F. cernua* extracts have also been investigated for application and evaluation in tomatoes (Salas-Méndez et al., 2019). Where the nanolaminate with *F. cernua* extract managed to extend the shelf life of tomato by reducing weight loss and microbial growth, as well as reducing gas exchange and ethylene production and maintaining firmness and color, making it an alternative to prolong the shelf life of tomato. In addition, an edible coating based on candelilla wax, whey protein, glycerol, and *F. cernua* extract has been formulated and optimized, which was applied to tomatoes, reducing weight loss and firmness in the fruit (Ruíz-Martínez et al., 2020). The sensory evaluation showed that the product obtained is acceptable to consumers. The edible coating added with *F. cernua* extract was the most effective in inhibiting the growth of pathogenic fungi. The visual appearance at the end of storage confirmed the beneficial effect of the edible coating.

CONCLUSIONS

F. cernua grows extensively in the arid zones of northern Mexico; it is not threatened in these ecosystems, nor is it currently exploited. The rational use of this non-timber forest resource would adhere to the Forestry Law. Its controlled use would only involve harvesting or pruning the upper third of the foliage, which would promote greater vigor and sprouting of the young buds that would later produce new branches, leaves, and more foliage, which could be used every 2 or 3 years. *F. cernua* extracts represent a natural source of bioactive phytochemicals, biodegradable and relatively non-toxic to humans, animals, and the environment. They are an eco-friendly alternative with great potential to combat insect pests (termites, *Sitophilus oryzae, Phthorimaea operculella*, and *Brevicoryne brassicae*), bacteria (*Pseudomonas cichorii, alternata, Mucor* sp., *S. sclerotorium*, and *C. gloesporioides*) that attack crops, as well as bacteria of clinical importance (H37Rv and CIBIN strains of *Mycobacterium tuberculosis*: UMF: 15: 99, *Enterobacter aerogenes, Escherichia coli, Salmonella typhi*, and *Staphylococcus aureus*). They also have hepatoprotective effects against ischemia-reperfusion injury in Wistar rats and anticancer, antiproliferative, and anti-inflammatory activity in A549 lung cancer cells and human breast cancer cells. In addition to having phytotoxic activity against the growth of *Amaranthus hypochondriacus* and *Echinochloa crus-galli*.

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