

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

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

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Resumen

El hojásén (*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 hojásén 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, Hojásén.

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, flourensadiol, 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	<i>Alterniasp.</i> , <i>Rhizoctonia solani</i> , and <i>Fusarium oxysporum</i>	(Jasso De Rodríguez et al., 2007)
Methanol: chloroform extract of leaves	4000 mg/L	<i>Colletotrichum gloesporoides</i>	(Guerrero Rodríguez et al., 2007)
Hexane leaf extract	4000 mg/L	<i>Colletotrichum gloesporoides</i>	(Guerrero Rodríguez et al., 2007)
Ethanolic extract of leaves	2000 mg/L	<i>Colletotrichum gloesporoides</i> and <i>Penicillium digitatum</i>	(Guerrero Rodríguez et al., 2007)
Leaf extract with	2000 mg/L	<i>Rhizoctonia solani</i>	(Castillo et al., 2010)

lanolin as a solvent	Leaf extract with cocoa butter as a solvent	1000 mg/L	<i>Rhizoctonia solani</i>	(Castillo et al., 2010)
Resin powder from aqueous extract of leaves	1,519 to 3,310 mg/L	<i>Rhizopus stolonifer</i> , <i>Botrytis cinerea</i> , <i>F. oxysporum</i> , and <i>C. gloesporioides</i>	(De León-Zapata et al., 2016)	
Ethanolic extract of leaves	2163 mg/L	<i>F. oxysporum</i> and <i>R. stolonifer</i> .	(Jasso de Rodríguez et al., 2017)	
Ethanolic extract of branches	4240 mg/L	<i>F. oxysporum</i> and <i>R. stolonifer</i>	(Jasso de Rodríguez et al., 2017)	
Ethanolic extract of leaves and branches	1692 mg/L	<i>R. stolonifer</i>	(Jasso de Rodríguez et al., 2017)	
Resin powder from aqueous extract of leaves	125 to 1000 mg/L	<i>F. oxysporum</i> , <i>B. cinerea</i> , <i>Penicillium sp.</i> , <i>Alternaria alternata</i> , <i>R. stolonifer</i> , <i>Mucor sp.</i> , <i>Sclerotinia sclerotorium</i> , and <i>C. gloesporioides</i>	(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. gloesporioides* (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. sclerotiorum*, 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 (Télez 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 $\mu\text{l L}^{-1}$ 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 $\mu\text{g L}^{-1}$ 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 $\mu\text{l ml}^{-1}$ 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. sclerotium*, 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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