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Phytochemical analysis of leaves and flowers extracts of *Psittacanthus Calyculatus* located in the Palenque hill of Purísima del Rincón, Guanajuato

Análisis fitoquímico de extracto de hojas y flores de *Psittacanthus Calyculatus* ubicado en el cerro del Palenque de Purísima del Rincón, Guanajuato

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ABSTRACT

The mistletoe *Psittacanthus calyculatus* affects several plant species in Mexico. In this study, the phytochemical profile and biological activity of mistletoe were analyzed. Two types of extraction were designed: methanol/acetone/water for leaf (E1) and flower (E2) and aqueous for leaf (E3) and flower (E4). The qualitative study showed phenols and flavonoids, among others. Total phenols were higher ($p < 0.03$) in E4 (32.84 ± 1.2 mg AGE/mL) than in E2, E1, and E3 (13.71 ± 1, 20.1± 0.3, 12.39 ± 0.4 mg AGE/mL, respectively). Flavonoids were higher in E4 (49.8 ± 2 mg EC/mL) ($p \leq 0.03$) than in E2, E1, and E3 (9.5 ±1, 20.4 ± 0.5 and 10.3 ± 5 mg EC/mL, respectively). DPPH and IC_{50} values measured antioxidant activity: E2 and E1 were 0.058 and 0.0035 mg/mL ($p < 0.03$) respectively, for E4 and E3 were 0.15 and 0.08 mg/mL ($p < 0.03$) respectively. The results suggest that this mistletoe possesses antioxidant activity because of its phytochemical content, which suggests that this plant could be an important source of natural nutrients with therapeutic activity.

KEY WORDS: Extract, *Psittacanthus calyculatus*, phytochemical, antioxidant, analysis.

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RESUMEN

El muérdago *Psittacanthus calyculatus* afecta a diversas especies vegetales en México. En este estudio se analizó el perfil fitoquímico y la actividad biológica de este muérdago. Se diseñaron dos tipos de extracción: metanol/acetona/agua para hoja (E1), flor (E2) y acuosa para hoja (E3), flor (E4). El estudio cualitativo mostró fenoles, flavonoides, entre otros. Los fenoles totales fueron mayores ($p < 0.03$) en E4 (32.84 ± 1.2 mg EAG/mL) que en E2, E1 y E3 (13.71 ± 1, 20.1± 0.3, 12.39 ± 0.4 mg EAG/mL, respectivamente). Los flavonoides fueron mayores en E4 (49.8 ± 2 mg EC/mL) ($p \leq 0.03$) que en E2, E1 y E3 (9.5 ±1, 20.4 ± 0.5 y 10.3 ± 5 mg EC/mL, respectivamente). La actividad antioxidante fue medida por DPPH y los valores de IC_{50}: E2 y E1 fueron 0.058 y 0.0035 mg/mL ($p < 0.03$) respectivamente, para E4 y E3 fueron 0.15 y 0.08 mg/mL ($p < 0.03$) respectivamente. Los resultados sugieren que este muérdago posee actividad antioxidante, esto debido al contenido de fitoquímicos, lo cual se sugiere que esta planta podría ser una importante fuente de nutrientes naturales, con actividad terapéutica.

PALABRAS CLAVE: Extracto, *Psittacanthus calyculatus*, fitoquímico, antioxidante, análisis.

Introduction

Members of the Loranthaceae family, specifically mistletoes, are hemiparasitic plants that acquire the necessary nutrients through parasitic association with a host (García-García *et al.,* 2021). Historically, mistletoes have been used for years as an alternative treatment for certain diseases through their application in traditional and folk medicine (Xie *et al.,* 2017). Phytochemical studies of this plant family have revealed significant therapeutic constituents such as lectins (Franz *et al.,* 1981; Wacker *et al.,* 2004), phenols and flavonoids (Luczkiewicz *et al.,* 2001; Vicaş *et al.,* 2011), terpenoids (Luczkiewicz *et al.,* 2001), steroids (Waly *et al.,* 2012), tannins (Torres *et al.,* 2019), and cardiotonic glycosides (Hlophe *&* Bassey, 2023). The Loranthaceae family has also been acknowledged for significant therapeutic properties, with its species being used for their antitumor (Park *et al.,* 1999), anti-inflammatory (Mothana *et al.,* 2012), antimicrobial (Egbuonu A. C. Cemaluk, 2012), antiviral (Lohézic-Le Dévéhat *et al.,* 2002), and antifungal effects (Xoca-Orozco *et al.,* 2022). In addition, these plants have been employed for treating hypertension, atherosclerosis, and cancer (Szurpnicka *et al.,* 2020).

The genus *Psittacanthus calyculatus* (*P. calyculatus)*, also known as the ‘true mistletoe’ belonging to the Loranthaceae family, is a hemiparasitic plant that thrives on various tree species in central and southern Mexico (Azpeitia *&* Lara, 2006). Due to the harm it causes to various tree

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species, it is considered a pest, parasitizing species such as *Acacia schaffneri* (Queijeiro-Bolaños *et al.,* 2020)*, Prosopis laevigata* (Quintana-Rodríguez *et al.,* 2018)*, Quercus desertícola* (Cuevas-Reyes *et al.,* 2017)*,* among others. Pharmacological studies of *P. calyculatus* describe its effects for treating arterial hypertension, vasodilation, and reduction of cholesterol and blood sugar levels; its anticancer activity has also been reported (Hernández Rodríguez *et al.,* 2015; Ibarra-Alvarado *et al.,* 2010; Bah *et al.,* 2011). Therefore, this study aimed to evaluate the phytochemical profile and biological activity of the mistletoe extract *P. calyculatus*, located in Cerro del Palenque of Purisima del Rincon Guanajuato Mexico, to identify compounds with significant therapeutic potential.

Material and Methods

Chemical reagents and solutions

Methanol (CH_3OH), hydrochloric acid (HCl), sodium carbonate (Na_2CO_3), ethanol (C_2H_6O), chloroform ($CHCl_3$), acetic anhydride ($C_4H_6O_3$), acetone (C_3H_6O) and sulfuric acid (H_2SO_4) were purchased from the brand J.T. BAKER®. Picric acid ($C_6H_3N_3O_7$), benzene (C_6H_6), ammonia (NH_3), Lead acetate ($Pb(C_2H_3O_2)_2$), copper sulfate ($CuSO_4$), sodium hydroxide (NaOH), Sudan reagent III, Mayer reagent, reagent Fehling, Benedict reagent and sodium nitrite ($NaNO_2$) were purchased from MEYER®. Iron (III) chloride ($FeCl_3$), gelatin, sodium chloride (NaCl), magnesium filings, glacial acetic acid (CH_3COOH), ninhydrin, formaldehyde (CH_2O), aluminum chloride ($AlCl_3$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Wagner reagent, Dragendorff reagent, Folin–Ciocalteu reagent, gallic acid and catechin were purchased from Sigma-Aldrich®. Baljet reagent was prepared with picric acid, ethanol, and sodium hydroxide.

Plant material

500 g of leaf (L) and flower (F) samples from *P. calyculatus* on mesquite (its host plant), were collected from the Cerro del Palenque natural protected area in Purísima del Rincón, Guanajuato, Mexico. Subsequently, they were dried in an Ecoshel HV-20 oven at 60°C with air circulation, following the recommendations of Naikwade, (2014), Sagrin & Chong, (2013), and Suchismita *et al.,* (2012), until reaching a constant weight. Each dried sample was ground using a Tapisa® industrial blender until a fine powder passed through an analytical sieve (mesh 150), aiming for a homogeneous contact surface. The resulting powder was stored in freezing conditions at -20°C until further use.

Extraction

Mistletoe extracts (ME) of *P. calyculatus* from the L and F samples were obtained by: solid-liquid extraction using methanol/acetone/water (MAW) and aqueous extraction (AE). In MAW the methodology proposed by Saura-Calixto *et al.,* (2007) was used; briefly, 5 g of each sample was added to CH_3OH acidified (8 mL L^{-1} HCl) - water (50-50 v/v) in a proportion of 50 mL g^{-1} of the sample, was subjected to constant stirring for 60 min at room temperature. It was then centrifuged at 5000 g (15 min at 25°C) and the phases were separated, keeping the supernatant at 4 °C. The

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precipitate was washed with an acetone-water mixture (70-30 v/v, 50 mL g^{-1} of sample) for 60 min, centrifuging under the same conditions, and the supernatants from each round of washing were combined. For this supernatant mixture, solvents were removed in a reduced atmosphere for 2 h at 60 °C, as recommended by Che Sulaiman *et al.,* (2017) and Zakaria & Kamal, (2016), for this purpose the DLAB® RE100-Pro rotary evaporator coupled to a Sego Vac® brand vacuum pump was used to generate reduced pressure, allowing the solvent to evaporate faster.

For the AE, the methodology proposed by Masangwa *et al.,* (2013), was employed, In brief, 5 g of each sample was combined with 200 mL of distilled water, maintained under constant agitation at room temperature for 24 h, centrifuged, and the solid particles were filtered. The water was removed using the same rotary evaporator under identical conditions. The obtained extracts were stored at 4°C in the dark until further use.

Phytochemical Analysis

Qualitative Evaluation of Phytochemical Compounds

This was determined through color estimation and precipitation. In each assay, a negative control (distilled and sterile water) was used, which did not contain ME to ensure color change. Each analysis, along with the negative control, was performed in triplicate.

Sesquiterpene Lactone Test

Baljet Test: 5 drops of the Baljet reagent were added to 4 mL of each ME. A color change in the ME from orange to red demonstrates the presence of sesquiterpene lactones (García-Granados *et al.,* 2019).

Saponin Tests (Steroidal and Triterpenic)

1 mL of each ME was mixed with 4 mL of distilled water and agitated in a vortex for 1 min. The appearance of foam at the top of the ME exceeding 2 mm in height, and if persistent for approximately 2 minutes, was considered positive (Bulugahapitiya, 2013).

Phenol Test

To 1 mL of each ME, 1 mL of C_2H_6O and 3 drops of $FeCl_3$ (5 %) were added. The following color changes in the ME indicate red-wine = general phenols, intense green = pyrocatechols, and blue = pyrogallols (Bulugahapitiya, 2013).

Test for Tannins and Pseudotannins

Precipitation with gelatin: 1 mL of each ME was combined with 5 mL of a gelatin solution (1 %) and NaCl (10 %). The test is positive if precipitation is observed, indicating high concentrations of tannins and pseudotannins (Bulugahapitiya, 2013).

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Flobatanin Test

1 mL of each ME was mixed with 3-4 drops of HCl (2 %). It is considered positive if a red precipitate appears in the ME (Phuyal *et al.,* 2019).

Flavonoid Test

Shinoda Test: To 1 mL of each ME, 0.1 g of magnesium shavings were added and placed in a water bath at 60 °C for 1 minute. Subsequently, 3-4 drops of concentrated HCl were added. The following color changes in the ME indicate red = aurones and chalcones, orange, red, or violet = flavones (Bulugahapitiya, 2013).

Alkaline Test: 1 mL of each ME was mixed with 1 mL of a NaOH solution (40 %), resulting in an intense yellow color. This test is positive if a decoloration is observed upon adding 1 mL of 10 % HCl (Bulugahapitiya, 2013).

Lead Acetate Test: 1 mL of each ME was added to 0.5 mL of ($Pb(C_2H_3O_2)_2$) (2 %). The appearance of a yellow color in the ME indicates the presence of these compounds (Bulugahapitiya, 2013).

Reducing Sugars Tests

Fehling Test: 1 mL of each ME was combined with 3-4 drops of Fehling's reagent. The appearance of a red color in the ME indicates the presence of reducing sugars (Khattak *et al.,* 2017).

Benedict Test: 1 mL of each ME was added with 3-4 drops of Benedict's reagent. The appearance of a brick-red color in the ME demonstrates the presence of reducing sugars (Khattak *et al.,* 2017).

Cardiotonic Glycosides Test

Keller-Kiliani Test: 1 mL of each ME was mixed with 1 mL of CH_3COOH, 3-4 drops of $FeCl_3$ (5 %), and 1 mL of concentrated (H_2SO_4). The test is positive if the formation of a brown ring at the interface is observed, along with the formation of a violet ring beneath the brown ring (Bulugahapitiya, 2013).

Quinones and Anthraquinones Test

Quinones

To 1 mL of each ME, 1 mL of concentrated H_2SO_4 was added. This test is positive if the formation of a red color is observed in the ME (García-Granados *et al.,* 2019).

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Anthraquinones

1 mL of each ME, 1 mL of C_6H_6, and 3-5 drops of an NH_3 solution (10 %) were mixed, the appearance of a red precipitate in the ME indicates the presence of these compounds (Archana *et al.,* 2012).

Coumarin Test

Fluorescence test: the mouth of the tubes containing the solutions of each ME (1mL) was covered with a circle of filter paper previously treated with NaOH (1N) and placed in boiling water for a few minutes. The filter paper was removed and examined under ultraviolet light. The appearance of fluorescence in the ME indicates the presence of these compounds (Bulugahapitiya, 2013).

Test for Phytosterols and Triterpenes

Liebermann-Burchard test: 1 mL of each ME was mixed with 1 mL of $CHCl_3$, 1 mL of $C_4H_6O_3$, and 3 drops of concentrated H_2SO_4. The formation of different colors in the ME indicates the presence of these compounds; The green color indicates the content of phytosterols, while the appearance of a pink to purple color indicates the content of terpenes and triterpenes (Bulugahapitiya, 2013).

Anthocyanin Test

Sulfuric acid test: 2 mL of each ME was mixed with 1 mL of concentrated H_2SO_4. This test is positive if an orange color appears on the ME interface (Agunos *et al.,* 2020).

Sodium Hydroxide test: 2 drops of NaOH (1N) were added to 2 mL of each ME. This test is positive if a blue or bluish-green coloration appears in the ME (Agunos *et al.,* 2020).

Betacyanin Test

2 mL of each ME was mixed with 3-5 drops of HCl (2 M), then placed in a water bath for 5 min and 3-5 drops of NaOH (2 M) were added. The presence of betacyanins in the ME is indicated by a change to yellow color (Harborne, 1998).

Protein and Amino Acid Test

Biuret: 1 mL of each ME was mixed with an equal volume of NaOH (40 %) and 2 drops of $CuSO_4$ (1 %). The appearance of violet color in the ME indicates the presence of proteins (Santhi & Sengottuvel, 2016).

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Ninhydrin: To 1 mL of each ME, 3 to 5 drops of freshly prepared ninhydrin reagent (0.2 %) were added and heated in a water bath. The appearance of a pink, purple, or blue color in the ME indicates the presence of amino acids (Santhi & Sengottuvel, 2016).

Test for Fatty Compounds

1 mL of each ME was mixed with 1 mL of Sudan reagent III. It was placed in a water bath until the solvent evaporated. The appearance of an orange color in the ME indicates the presence of these compounds (Godlewska *et al.,* 2022).

Alkaloid Test

Wagner: 1 mL of each ME was mixed with 1 mL of HCl (1 %) and 5 drops of cold Wagner reagent. A prominent reddish-brown precipitate in the ME indicates that the test is positive for these compounds (Khattak *et al.,* 2017).

Mayer: 2-3 drops of Mayer's reagent were added to 1 mL of each ME. The appearance of turbidity or a yellow precipitate in the ME indicates that the test is positive for these compounds (Bulugahapitiya, 2013).

Marquis: 1 mL of each ME was mixed with 2 mL of concentrated H_2SO_4, 1 mL of Marquis reagent, and 3 drops of CH_2O (40 %). The appearance of a violet (purple) color in the ME is indicative of the presence of opioid derivatives (Santhi & Sengottuvel, 2016).

Dragendorff: 1 mL of each ME was mixed with 1 mL of HCl (1 %) and 3-4 drops of Dragendorff reagent, the presence of alkaloids in the ME is positive if there is an orange, reddish or yellow precipitate (Khattak *et al.,* 2017).

Test for Total Cyanogenic Glycosides (Toxic compounds)

Picrate Paper Analysis Method

For this test, the methodology described by Appenteng *et al.,* (2021) was used. Briefly, the picrate paper was prepared by wetting a sheet of Whatman 1 filter paper in a picrate solution (1.4 %) w/v diluted in a Na_2CO_3 solution (2.5 %) w/v), air-drying the paper, and cutting 5 cm x 1 cm strips. 2 mL of each ME and 1 mL of $CHCl_3$ were added to a test tube. The paper strips were placed at a distance of 1 cm from the sample, taking care that they did not touch the walls of the tube. They were heated in a water bath for 30 min at 100 °C. This test is positive for the appearance of a pink to red color on the picrate paper strip.

Spectrophotometric Quantification of Total Phenols

It was determined using Folin-Ciocalteu, following the procedure of Pallag *et al., (*2016) and Vicaş *et al.,* (2011). Briefly, 25 µL of each ME was placed in the wells of an-ELISA microplate,

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80 µL of distilled H_2O, 5 µL of Folin-Ciocalteu reagent, and 80 µL of Na_2CO_3 (7.5%) were added. The mixture was allowed to stand for 30 minutes in the dark and the absorbance was measured at 760 nm on a Thermo Scientific™ multiskan microplate spectrophotometer. The content of total phenols was calculated in mg equivalents of gallic acid per mL of extract (mg EAG/mL), for which a calibration curve was constructed between 0-0.25 mg/mL.

Spectrophotometric Quantification of Total Flavonoids

The colorimetric technique was used using aluminum chloride, proposed by Miere *et al.,* (2021). Briefly, 20 µL of each ME was placed in wells of an-ELISA microplate, 6 µL of $NaNO_2$ (5 %), 12 µL of $AlCl_3$ (10 %), 122 µL of distilled H_2O were added, allowed to stand for 6 min, and 40 µL of freshly prepared NaOH (1 M) was added. The absorbance was measured at 510 nm in the same spectrophotometer equipment mentioned. The total concentration of flavonoids was expressed as mg catechin equivalents per mL of extract (mg EC/mL), for which a standard curve was constructed between 0-0.1 mg/mL.

Determination of Antioxidant Activity with DPPH

The antioxidant activity of ME was determined using the DPPH method described by Kleszken *et al.,* (2022). Briefly, the reaction mixture was prepared using 200 µL of DPPH (0.1 mM) with CH_3OH (80 %) (blank) and 40 µL of each ME at different concentrations (0.05, 0.1, 0.25, 0.5, and 1 µg/mL). It was left to rest for 30 min in the dark and at room temperature. The absorbance was recorded at 517 nm in the spectrophotometer mentioned above. The experiment was performed in triplicate. Capture activity was calculated using the following equation (Budau *et al.,* 2022):

$$\%Radical\ scavenging\ activity\ DPPH = [(A0 - A1) \div A0] \times 100$$

Where:

A0= Absorbance of DPPH in its radical form (blank)

A1= Absorbance of DPPH with ME after 30 min of reaction

The IC_{50} factor was calculated, which is defined as the required concentration of extract in mg EAG/mL of sample that is required to inhibit 50 % of DPPH free radicals. This value was obtained through linear regression of the percentage of capture versus concentration of each ME (Msaada *et al.,* 2017). The lower the IC_{50} value, the more powerful the substance will be to eliminate DPPH, therefore, this implies a greater antioxidant activity (Olugbami *et al.,* 2014).

Statistical analysis

All experiments were performed in triplicate and the data obtained were analyzed with the statistical package IBM SPSS STATISTICS 25. Linear regression analysis and one-way

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ANOVA were applied. A comparison test of means was performed using the Tukey test. Data were expressed as ± standard deviation. $p < 0.05$ was considered the level of statistical significance.

Results and discussion

Qualitative analysis of phytochemical compounds

The extracts obtained for each plant tissue of *P. calyculatus* were methanol/acetone/water leaf (E1), methanol/acetone/water flower (E2), aqueous leaf extraction (E3) and aqueous flower extraction (E4). The phytochemical content evidenced the presence of sesquiterpene, lactones, saponins, phenols, tannins, pseudo tannins, flavonoids, betacyanin, cardiotonic glycosides, fatty compounds, anthraquinones, coumarins, triterpenes, anthocyanins and alkaloids (Table 1). The phytochemical compounds phenols, flavonoids, and anthocyanins identified in *P. calyculatus* have been reported by Ochoa-Cruz *et al.,* (2023), Serrano-Maldonado *et al.,* (2011), and Reynoso Silva *et al.,* (2022), who carried out a phytochemical study of fruit and leaf, the content of alkaloids has also been evidenced in the same species of mistletoe (Bah *et al.,* 2011).

Table 1. Qualitative analysis of phytochemical compounds in extracts of *P. calyculatus*

Phytochemical compounds	Chemical test	E1	E2	E3	E4
Sesquiterpene lactones	Baljet	++	++	++	++
Saponins (steroidal and triterpenic)	Vortex agitation	+++ (Both)	+++ (Both)	+++ (Both)	+++ (Both)
Phenols	Ferric chloride (5 %)	+++	+++	+++	+++
Tannins and pseudotannins	Precipitation with gelatin	+++	+	+++	+
Flobatanins	HCl 2 %	++	-	++	-
Flavonoids	Shinoda	++	+	+++	++
	Alkaline	++	++	++	+
	Lead acetate	++	+	++	++
Anthocyanins	Concentrated H_2SO_4	-	-	-	-
	NaOH 1N	-	-	-	-
Betacyanins	HCl (2M) y NaOH (2M)	++	++	++	++

Content: abundant (+++), moderate (++), low (+), absent (-); E1: methanol/acetone/water extraction of leaves, E2, methanol/acetone/water extraction of flowers, E3: aqueous extraction of leaves, E4: aqueous extraction of flowers.

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Continuation

Table 1. Qualitative analysis of phytochemical compounds in extracts of *P. calyculatus*

Phytochemical compounds	Chemical test	E1	E2	E3	E4
Reducing Sugars	Fehling	-	-	-	-
	Benedict	-	-	-	-
Cardiotonic glycosides	Keller-Kilani	+++	+++	+++	+++
Quinones	Concentrated H_2SO_4	-	-	-	-
Anthraquinones	NH_4OH 10 %	+	+	+	+
Coumarins	NaOH 1N and filter paper	+	+	++	+
Fatty compounds	Sudan III	++	++	++	++
Phytosterols and triterpenes	Liebermann-Burchard	++ (Only Triterpenes)	++ (Only Triterpenes)	++ (Only Triterpenes)	++ (Only Triterpenes)
Aminoacids	Ninhydrin 0.2 %	-	-	-	-
Proteins	Biuret	-	-	-	-
Alkaloids	Wagner	+	+++	+++	+++
	Mayer	+	+	+	+
	Erdman	-	-	+	+
	Marquis	-	-	++	++
	Dragendorff	++	++	++	++
Cyanogenic glycosides	Sodium picrate and chloroform	-	-	-	-

Content: abundant (+++), moderate (++), low (+), absent (-); E1: methanol/acetone/water extraction of leaves, E2, methanol/acetone/water extraction of flowers, E3: aqueous extraction of leaves, E4: aqueous extraction of flowers.

There are several studies of other mistletoe species belonging to the same family, *Loranthaceae*, in which phytochemical compounds similar to those in Table 1 have been reported, such as *Loranthus micranthus Linn, Viscum continuum E. Mey, Phoradendron bollanum,* and *Viscum album subs. Austriacum* (García-García *et al.,* 2021; Hlophe *&* Bassey, 2023; Mapfumari *et al.,* 2022).

Quantitative analysis of phenols

The concentration of total phenols was calculated with the help of the graph shown in Figure 1A, and the equation of the resulting curve was y= 0.0041x+0.0799, where R^2 =0.9988. It was observed that E4 contains a higher concentration of total phenols compared to E2. On the other hand, in E1, it presented a more significant amount of phenolic compounds than in E3 (figure 1 B).

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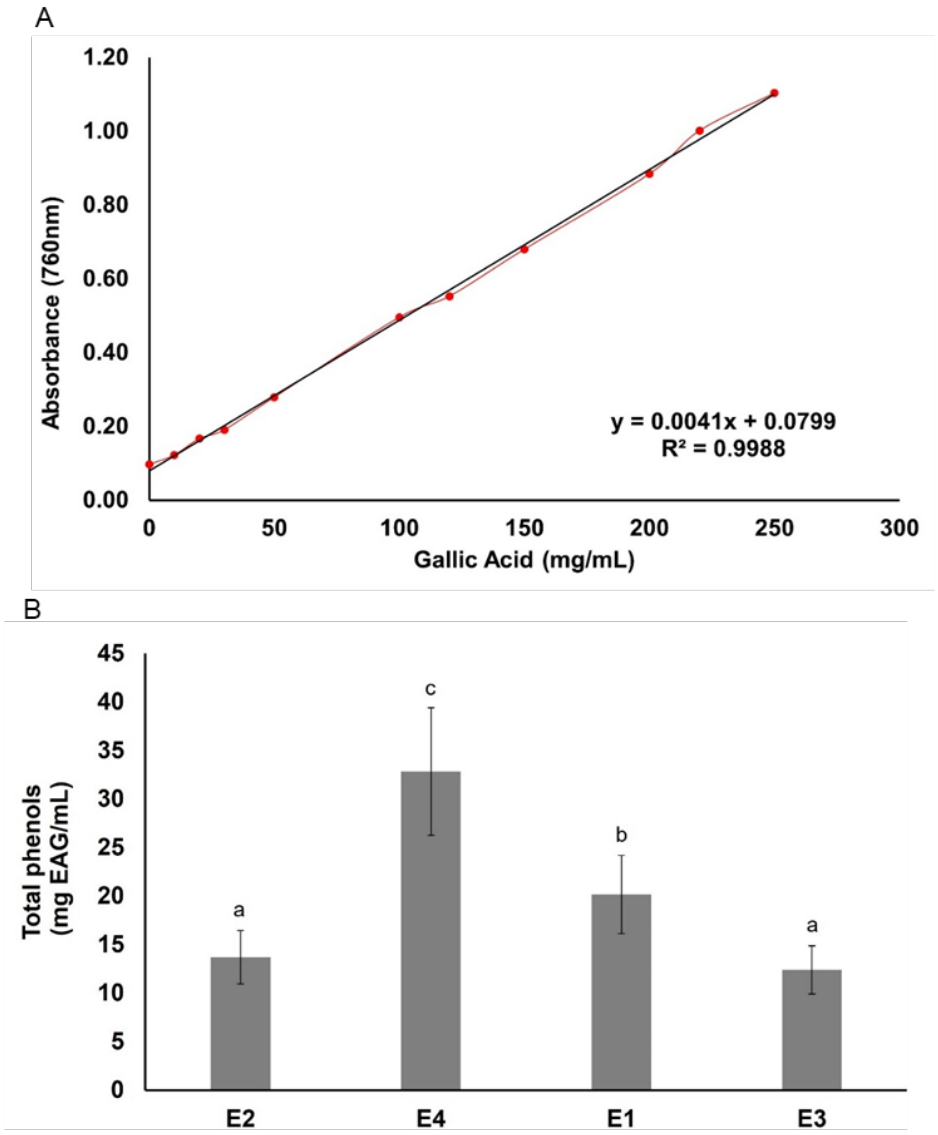

Figure 1. Concentration of total phenols. (A) Gallic acid standard curve. (B) Concentration of total phenols in the extracts.

Different letters indicate significant differences ($p < 0.03$).

Phenols have been reported to exhibit excellent antioxidant activity due to the presence of hydroxyl groups that act as hydrogen donors, which allows them to have REDOX properties and thus act as reducing agents (Wintola *&* Afolayan, 2011). Published studies of *P. calyculatus* have observed a high concentration of total phenols in stems, leaves, flowers (Ibarra-Alvarado *et al.,*

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2010; Reynoso Silva *et al.,* 2022) and fruit (Ochoa-Cruz *et al.,* 2023). Different species of mistletoe from the Loranthaceae family, such as *Phragmanthera capitata* (Ohikhena *et al.,* 2018), *Tristerix tetrandus Mart* (Simirgiotis *et al.,* 2016) and *Dendrophthoe pentandra* (Alharits *et al.,* 2019) have shown a high content of total phenols in leaves and flowers.

Quantitative Analysis of Flavonoids

The concentration of flavonoids was calculated with the help of the graph shown in Figure 2A and with the equation of the curve y= 0.0017x+0.0414, where R^2= 0.9951. The results showed that E4 showed a greater amount of total flavonoids than E2. The leaf presented a greater amount of total flavonoids in E3 than in E1.

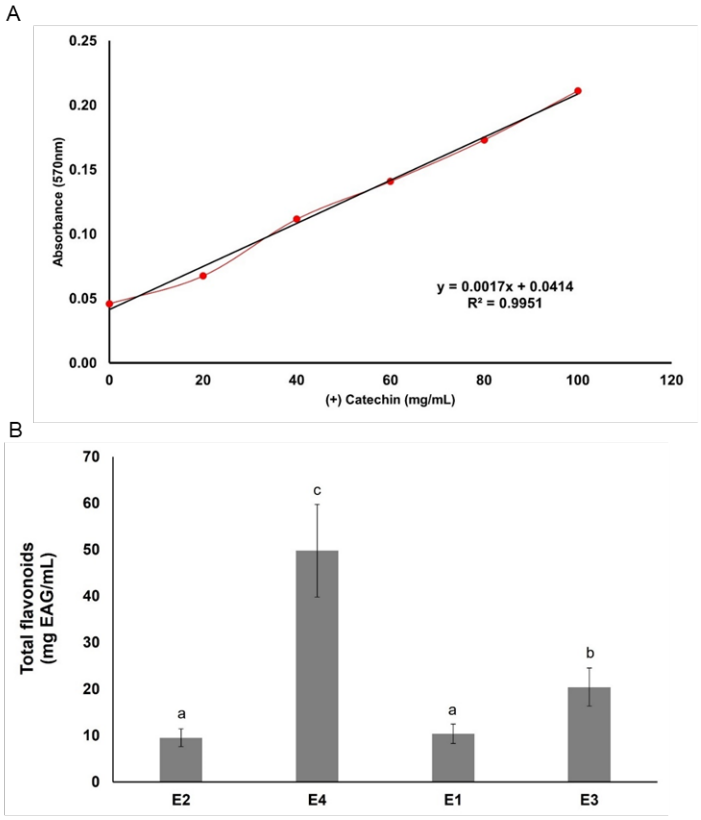

Figure 2. Concentration of total flavonoids. (A) Catechin standard curve. (B) Concentration of total flavonoids in the extracts.

Different letters indicate significant differences ($p < 0.03$).

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Flavonoids are secondary metabolites produced by plants and represent a large group of phenolic compounds; in addition, critical biological activities such as antioxidants, anticancer, anti-inflammatory, antithrombotic, and antimicrobial have been attributed to them (Yao *et al.,* 2013).

Recent reports on *P. calyculatus* have reported the flavonoid content in leaves (Reynoso Silva *et al.,* 2022) and fruits (Ochoa-Cruz *et al.,* 2023). Until now, there are no reports of flavonoid content in *P. calyculatus* flowers, but other mistletoe species of the Loranthaceae family have demonstrated a high content of these compounds: *Tristerix tetrandus Mart* and *Dendrophthoe pentandra* (Alharits *et al.,* 2019; Simirgiotis *et al.,* 2016).

Antioxidant Activity

The DPPH free radical scavenging method is widely used to evaluate the antioxidant activity of natural compounds and plant extracts (Do *et al.,* 2014). Radical scavenging is very important to prevent free radical damage in different diseases (Hlophe & Bassey, 2023). it was observed that all *P. calyculatus* extracts evidenced DPPH scavenging activity (%) and low IC_{50} values (Table 2).

Table 2. DPPH scavenging activity and IC_{50}

Extract	% DPPH removal	IC_{50} (mg/mL)	Regression	R^2
E2	56.71 ± 12.01^{a,b}	0.058	y = 125.62x + 42.641	0.9528
E4	45.89 ± 10.92^{b,c}	0.15	y= 106.92x + 33.925	0.8352
E1	63.06 ± 13.15^{a,b}	0.0035	y = 125.98x + 49.559	0.9879
E3	53.19 ± 9.76^{c}	0.08	y = 102.06x + 41.768	0.9525

Mean values ± standard deviation. Different letters indicate significant differences ($p < 0.03$).

E2 and E1 showed a higher percentage of DPPH uptake and low IC_{50} values compared to E4 and E3. Reports on *P. calyculatus* have observed DPPH uptake activity in fruit extract (Ochoa-Cruz *et al.,* 2023), stems, leaves, and flowers (Ibarra-Alvarado *et al.,* 2010). Other mistletoe species belonging to the family Loranthaceae have shown DPPH uptake in L and F extract: *Phragmanthera capitata* (Ohikhena *et al.,* 2018), *Tristerix tetrandus* (Simirgiotis *et al.,* 2016), *Loranthus micranthus* (Hlophe & Bassey, 2023) y *Viscum álbum* (Hong *et al.,* 2015; Kleszken *et al.,* 2022; Orhan *et al.,* 2014).

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Conclusions

This study revealed that Mexican Mistletoe *P. calyculatus* is a great source of important phytochemical compounds, including alkaloids, phenols, and flavonoids, which act as antioxidant agents. This plant has great potential for future use in the treatment of diseases related to oxidative stress, but further research is required through studies that determine its identification, separation, and quantification of individual phytochemical compounds, to support the application of this Mexican plant in the field of health.

Authors' contribution

Methodology development Sánchez-Guevara, D. and Hernández Mendoza, G.; Experimental validation Xoca-Orozco, L.A. and Flores-Sierra, J.J.; Results analysis Reyes-Bautista, R. and Hernandez Mendoza, G.; Data management, Sanchez-Guevara, D. and Xoca-Orozco, L.A.; Manuscript writing and preparation, Flores-Sierra, J.J. and Reyes-Bautista, R.; Writing, revising and editing, Hernández Mendoza, G., Xoca-Orozco, L.A. and Reyes-Bautista, R.; Project manager, Flores-Sierra, J.J.

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Conflict of interest

"The authors declare that they have no conflicts of interest".

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