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Phenolic and volatile compounds, total protein content, antioxidant and antidiabetic activities of leaves of *Palicourea padifolia* (Rubiaceae)

Compuestos fenólicos y volátiles, contenido total de proteínas, actividad antioxidante y antidiabética de las hojas de *Palicourea padifolia* (Rubiaceae)

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Abstract

Background and Aims: Information on the chemical composition and biological activities of *Palicourea padifolia* is scarce. This study aimed to 1) measure the total content of nutrients, macroelements, proteins, and secondary metabolites, 2) conduct metabolomic analyses on phenolic and volatile compounds, and 3) assess the antioxidant and antidiabetic activities from the leaves of this species.

Methods: The total content of nutrients and macroelements was determined by spectroscopic, spectrometric, and spectrophotometric approaches. The total content of alkaloids, terpenes, phenols, flavonoids, tannins, and proteins was determined by spectrophotometric approaches. Volatile and phenolic compounds were identified by gas and liquid chromatography, respectively, coupled to mass spectrometers. The antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, and the antidiabetic potential was determined by the inhibition of α -glucosidase.

Key results: The leaves of *P. padifolia* were rich in Ca, Na, and Mg, and exhibited a low protein content. The leaf methanolic extract (LME) contained high levels of alkaloids, phenols, and terpenes, while flavonoids were present at low concentrations. The major phenolic compounds identified were chlorogenic acid, scopoletin, *trans*-cinnamic acid, and (-)-epicatechin. In addition, a leaf petroleum ether extract (LPE) contained mainly phytol, palmitic acid, (Z)-7-tetradecenal, octadecanoic acid and linoleic acid. The leaves exhibited a high antioxidant capacity, inhibiting DPPH and ABTS radicals, and the LME showed low inhibition of α -glucosidase enzyme.

Conclusions: *Palicourea padifolia* leaves had a rich composition of cations such as Ca, Na, and Mg. In addition, the LME contained alkaloids, terpenes, lipids, and phenolics, which support the potent antioxidant capacity. In contrast, the LME exhibited a low inhibition of the α -glucosidase enzyme.

Key words: antioxidants, gas chromatography, mass spectrometry, phytochemistry, specialized metabolites, wax flower.

Resumen

Antecedentes y Objetivos: Existe poca información sobre la composición química y las actividades biológicas de *Palicourea padifolia*. Este estudio tuvo como objetivos 1) medir el contenido total de nutrientes, macroelementos, proteínas y metabolitos secundarios, 2) realizar análisis metabolómicos de compuestos fenólicos y volátiles, y 3) evaluar las actividades antioxidantes y antidiabéticas de las hojas de esta especie.

Métodos: El contenido total de nutrientes y macroelementos se determinó mediante métodos espectroscópicos, espectrométricos y espectrofotométricos. El contenido total de alcaloides, terpenos, fenoles, flavonoides, taninos y proteínas se determinó mediante métodos espectrofotométricos. Los compuestos volátiles y fenólicos se identificaron mediante cromatografía de gases y líquidos, respectivamente, acoplados a espectrómetros de masas. La actividad antioxidante se determinó mediante los ensayos con 2,2-difenil-1-picrilhidracilo (DPPH) y ácido 2,2'-azino-bis(3-etilbenzotiazolina-6-sulfónico) (ABTS), y el potencial antidiabético se determinó mediante la inhibición de la α -glucosidasa.

Resultados clave: Las hojas de *P. padifolia* fueron ricas en Ca, Na y Mg, y presentaron un bajo contenido de proteínas. El extracto metanólico de hoja (LME) contenía altos niveles de alcaloides, fenoles y terpenos, mientras que los flavonoides estuvieron presentes en bajas concentraciones. Los principales compuestos fenólicos identificados fueron el ácido clorogénico, la escopoletina, el ácido transcínico y la (-)-epicatequina. Además, un extracto de éter de petróleo de hoja (LPE) contenía principalmente fitol, ácido palmítico, (Z)-7-tetradecenal, ácido(Z)-octadecanoico y ácido linoleico. Las hojas exhibieron alta capacidad antioxidante, inhibiendo los radicales DPPH y ABTS y el LME mostró baja inhibición de la enzima α -glucosidasa.

Conclusiones: Las hojas de *Palicourea padifolia* tuvieron una rica composición de cationes como Ca, Na y Mg. Además, el LME contenía alcaloides, terpenos, lípidos y fenólicos, que respaldan la potente capacidad antioxidante. Por el contrario, el LME mostró una baja inhibición de la enzima α -glucosidasa.

Palabras clave: antioxidantes, cromatografía de gases, espectrometría de masas, fitoquímica, flor de cera, metabolitos especializados.

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Introduction

The family Rubiaceae is a promising source of novel bioactive compounds, given the large number of described natural products, their structural diversity, and their reported pharmacological activities for different species (Heitzman et al., 2005). Several members of this family are widely used in traditional medicine, and some exhibit antibacterial and antiviral properties, as well as antioxidant activity (De Moura et al., 2020b). Novel compounds (i.e., alkaloids) were found in some species (Martins and Nunez, 2015).

In this context, a potential source of bioactives is *Palicourea* Aubl., a genus of this family, which comprises nearly 700 species of shrubs and small trees that are widespread in humid tropical regions (WFO, 2024). Extracts or fractions of aerial parts of certain *Palicourea* species have been used as traditional medicine for various ailments (Formagio et al., 2022). For example, aerial parts and the roots of *Palicourea coriacea* (Cham.) K. Schum. have been used to treat renal disorders (Freitas et al., 2011). The methanolic extract, non-alkaloid, and alkaloid fractions of *Palicourea minutiflora* (Müll. Arg.) C.M. Taylor exhibit anti-inflammatory effects and show potential anticancer activity *in vitro* (De Moura et al., 2020a). The leaves of *Palicourea tomentosa* (Aubl.) Borhidi have been used in palliative treatments to relieve inflammation and pain due to their analgesic, anti-inflammatory, and anti-acetylcholinesterase properties (Formagio et al., 2022). Similarly, the leaf extract of *Palicourea rigida* Kunth is recognized to have anti-inflammatory and anti-edematogenic properties (Alves et al., 2016).

Palicourea species exhibit a diverse and complex phytochemistry, characterized by specialized alkaloid-type metabolites with considerable structural diversity (Berger et al., 2015, 2017; Formagio et al., 2022). Alkaloids have been reported in *P. coriacea*, including β -carboline lyaloside

(Valverde et al., 1999), strictosidinic acid, *epi*-strictosidinic acid, and strictosidinic ketone and calycanthine (Do Nascimento et al., 2006). Tryptamine-iridoid alkaloid lagamboside has been reported in *Palicourea acuminata* (Benth.) Borhidi (Berger et al., 2012), while in *Palicourea crocea* (Sw.) Roem & Schult. new alkaloids from the loganine and secologanine pathway were isolated (Berger et al., 2015). Triterpenes, including betulinic acid and lupeol, diterpene phytol, and iridoid glycosides, such as sweroside and secaxiloganin, have been successfully isolated from *P. rigida* (Alves et al., 2016).

Compound bioprospecting in *Palicourea condensata* Standl. has led to the discovery of new plant peptides with antiviral potential, such as palicurein, which has potential pharmacological use for anti-HIV activity (Bokesch et al., 2001). In addition, extracts and fractions of *Palicourea hoffmannseggiana* (Roem. & Schult.) Borhidi have shown antiparasitic activity against resistant strains of *Plasmodium falciparum* Welch 1897, the organism that causes malaria in humans (Ohashi et al., 2021). All these studies hold great promise for discovering novel compounds in *Palicourea* species that could be used in the pharmaceutical industry or biotechnology (Sandra et al., 2018). However, some species, including *Palicourea croceoides* Desv. ex Ham., *Palicourea marcgravii* A. St.-Hil., *Palicourea aeneofusca* (Müll. Arg.) Standl., *Palicourea juruana* K. Krause and *Palicourea grandifolia* (Humb. & Bonpl. ex Roem. & Schult.) Standl. have been reported to exhibit phytotoxicity (Cook et al., 2014; Lee et al., 2014).

Additionally, *Palicourea padifolia* (Humb. & Bonpl. ex Roem. & Schult.) C.M. Taylor & Lorence, locally known as “*flor de cera*” or “*flor de mayo*”, is a species widely distributed on the Atlantic slope of Mexico from Hidalgo and north of Oaxaca, as well as in the Los Tuxtlas volcanic massif in Veracruz, and extending from Chiapas in Mexico to

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Panama (Gutiérrez-Rodríguez et al., 2011). This species is a shrub that grows to a height of 4-8 m with leaves of 6-15 cm long and 2-5 cm wide (Taylor, 1989). The shrub produces reddish-purple pyramidal inflorescences with yellow flowers and fleshy, purplish-black fruits (Taylor, 1989) (Fig. 1).

Palicourea padifolia has an ornamental use (Leszczynska-Borys, 1995) as well as an ecological value as a food resource and soil stabilizer (Contreras and Ornelas, 1999; Williams-Linera and Herrera, 2003). The flowers are pollinated by small bees, bumblebees, butterflies, and hummingbirds (Hernández-Ramírez, 2018). The fruits are a food source for several species of birds (Ree, 1997; Contreras and Ornelas, 1999; Ornelas et al., 2004). Research on *P. padifolia* has primarily concentrated on its reproductive

ecology (González et al., 2004; Ornelas et al., 2004) and microsatellite markers (Covarrubias et al., 2011). This species does not appear to be used in Mexican traditional medicine, and literature about the chemical composition and information of the biological activities of *P. padifolia* leaves is scarce. Alkaloids such as strictosidine, lyaloside and (E)-O-(6')-(4"-hydroxy-3",5"-dimethoxy)-cinnamoyl lyaloside have been isolated in *P. padifolia* (Berger et al., 2015). In addition, the compound kaempferol 3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside has also been identified in *P. padifolia* (Berger et al., 2016). According to Ramírez-Reyes et al. (2019), the methanolic leaf extracts did not exhibit inhibitory activity *in vitro* against phytopathogenic bacteria, including *Chryseobacterium* sp., *Pseudomonas cichorii* (Swingle 1925) Stapp 1928, *Pectobacterium*



Figure 1: *Palicourea padifolia* (Humb. & Bonpl. ex Roem. & Schult.) C.M. Taylor & Lorence structures. A. leaves; B. yellow flowers; C. mature purplish black fruit.

carotovorum (Jones 1901) Waldee 1945, and *Pantoea stewartii* (Smith 1898) (Mergaert et al., 1993).

Due to the lack of knowledge about the phytochemistry and biological activity of *P. padifolia* and given the historical medicinal applications of several *Palicourea* species, it is postulated that *P. padifolia* leaves might have secondary metabolites with properties like antioxidant activities, or antidiabetic potential. The objectives of this study are to 1) determine in the leaves of this species the total content of carbon (C), nitrogen (N) sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), protein, alkaloids, terpenes, phenols, and flavonoids, 2) evaluate the content of phenolic and volatile compounds by a metabolomic analysis of leaf methanolic extract (LME) and the leaf petroleum ether extract (LPE), respectively; and 3) assess the antioxidant and antidiabetic capacity of the LME.

Materials and Methods

Plant material

Fresh young leaves of *Palicourea padifolia* (500 g) were collected from three separate individuals located in the Francisco Javier Clavijero Botanical Garden and in the cloud forest sanctuary of the Instituto de Ecología, A.C. (19°30'51.5"N, 96°56'32.31"W), Xalapa, Veracruz, Mexico. The identification of plant material was carried out by the curators of the herbarium XAL of the Instituto de Ecología, A.C. (Thiers, 2024), and a single specimen was deposited in the same herbarium (voucher # XAL0106254).

Elemental analysis

The total content of C and N was determined from 1 g of freeze-dried leaves (n=3) by dry combustion using an auto-analyzer (TruSpec CN, LECO, Corporation, St. Joseph, USA). The quantification of P, Na, K, Ca, and Mg was performed according to Etchevers (1988). The content of Ca and Mg was quantified by atomic absorption using a fast-sequential atomic absorption spectrometer (AA240FS, Varian, American Laboratory Trading, San Diego, USA), while the K and Na content was determined by flame spectrophotometry (410 Corning, Sherwood Scientific Ltd, Cambridge, UK). For the quantification of total P, the colorimetric method of vanadomolybdophosphoric acid was

employed using a spectrophotometer (Genesys 20, Thermo Scientific, Waltham, USA).

Total content of alkaloids, terpenes, phenols, and flavonoids

Spectrophotometric methods were applied to the methanol crude extracts of leaves obtained from three individuals of this plant species. The total content of alkaloids and flavonoids was determined using the method of Tambe and Bhambar (2014), while the total phenolics content was calculated following the methodology of Singleton et al. (1999). The method described by Borgonetti et al. (2020) was used to estimate the total terpenes content.

Determination of total protein content

Protein content was determined utilizing the Biuret method (Parvin et al., 1965). A homogeneous sample of leaf powder weighing 1 g was combined with 20 ml of distilled water. Next, 9.5 ml of 26.6% sodium sulfate was added, mixed, and diluted to a total volume of 50 ml. The mixture was then allowed to rest for 10 min. Subsequently, 2 ml of the mixture was added to 8 ml of Biuret reagent and inverted before resting for 30 minutes at room temperature in dark conditions. The absorbance was measured at $\lambda=540$ nm with a UV/Vis spectrophotometer (Jenway, Model 6305, Stone, UK). Albumin was used to generate a standard curve.

Identification and quantification of phenolic compounds by UPLC-QqQ analysis

Leaf methanolic extract (LME) was obtained using an accelerated solvent extraction system (ASE 350, Dionex Corporation, Sunnyvale, USA), following the protocol previously described by Infante-Rodríguez et al. (2020). For this purpose, 3 g of plant material was mixed with 1 g of diatomaceous earth (Thermo Scientific, Waltham, USA) and placed in a 34 ml cell. This method consisted of a single static cycle of 5 min at 60 °C. The methanolic extract was concentrated by rotary evaporation under reduced pressure at 40 °C (R11, Büchi, Flawil, Switzerland). The identification and quantification of phenolic compounds was performed in an Ultra Performance Liquid Chromatograph coupled



to a triple quadrupole mass spectrometer (UPLC-QqQ, Agilent Technologies 1290-6460, Santa Clara, USA), using a dynamic multiple reaction monitoring (dMRM) method following the procedure previously reported (Infante-Rodríguez et al., 2020).

Identification of volatile compounds by GC-MS analysis

The leaf petroleum ether extract (LPE) for Gas Chromatography coupled to Mass Spectrometer (GC-MS) analysis was obtained using the method of Borgonetti et al. (2020). An aliquot of 10 g of lyophilized vegetable powder was taken and soaked in methanol for 24 h. Subsequently, the filtrate was extracted with petroleum ether; the LPE was analyzed using a GC coupled to a single quadrupole MS (Shimadzu 2010 Plus-QP2010 Ultra, Tokyo, Japan), equipped with a ZB-5MSi column (30 m × 0.25 mm ID × 0.25 µm). Electron impact (70 eV) spectra were obtained. Helium was the carrier gas (0.8 cm³/min, constant flow), and a splitless injector (temperature of 250 °C, split valve delay of 3 min) was used to insert the sample. The oven temperature was held at 50 °C for 2 min, then programmed to increase at a rate of 15 °C/min to 280 °C, which was maintained for 10 min. The ion source temperature was 250 °C.

Tentative identifications were made by comparison of fragmentation patterns with those patterns available in the NIST/EPA/NIH Mass Spectral Library, NIST 11 software v. 2.0 (NIST, 2019). Some identifications were confirmed by comparison of chromatographic retention times and mass spectra with those of commercially available standards analyzed in the same instrument and analytical conditions using a range of 84-100% similarity values, with the GC-MS solutions software v. 2.72 (Shimadzu, Tokyo, Japan).

Antioxidant activity

Determination of DPPH radical inhibition percentage

The method for determining DPPH radical inhibition was previously reported by Brand-Williams et al. (1995) and modified by Juárez-Trujillo et al. (2018). An aliquot of the LME (30 µl) was added to 96-well plates, followed by the DPPH reagent to achieve a final volume of 300 µl. The

mixture was incubated in darkness for 30 min. Similarly, controls and blanks were prepared for each extract. Absorbance measurements at λ=517 nm were performed by triplicate in a spectrophotometer (Multiskan FC model IVD, Marsiling Industrial Estate, Singapore). To determine the percentage of free radical inhibition, the following equation was employed:

$$\text{DPPH radical inhibition (\%)} = (1 - ((\text{sample absorbance} - \text{blank absorbance}) / \text{control absorbance})) \times 100$$

Determination of antioxidant activity by ABTS assay

Antioxidant activity was also evaluated by ABTS assay (Re et al., 1999; Juárez-Trujillo et al., 2018). For this, 30 µl of extract and 270 µl of ABTS reagent were added and subsequently incubated at 25 °C for 30 min. Next, the absorbance was measured at λ=734 nm (Multiskan FC model IVD, Marsiling Industrial Estate, Singapore). ABTS reagent (300 µl) was used as blank. A calibration curve was made using a solution prepared by dissolving 10 mg of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) in 5 ml of methanol and 5 ml of distilled water. Subsequently, dilutions were made to known concentrations between 0.1 and 1 mg/ml, and results were expressed in mg equivalents of Trolox.

α-Glucosidase enzyme inhibition assay

The α-glucosidase inhibition by the LME was determined by *in vitro* enzymatic inhibitory assay according to Infante-Rodríguez et al. (2022). The α-glucosidase enzyme (≥100 U/mg protein) from the yeast *Saccharomyces cerevisiae* (Desm.) Meyen (Sigma Aldrich St. Louis, USA) was diluted to 0.005 mg/ml in phosphate buffer (PB, 100 mM, pH 7.2, Sigma Aldrich, St. Louis, USA). Then, 20 µl of LME dissolved at 1 mg/ml in PB was mixed with 100 µl of 4-nitrophenyl-α-D-glucopyranoside (Sigma-Aldrich, St. Louis, USA) at 1 mM in PB. Acarbose (Sigma-Aldrich, St. Louis, USA) was used as a positive control (30 mM). The reaction mixture was incubated for 5 min at 30 °C. After the incubation time, the enzyme was added to each well, and the microplate was incubated for 30 min at 30 °C. Absorbance was measured at λ=405 nm at the beginning and after 30 min



in a microwell spectrophotometer (Multiskan FC, Thermo Scientific, Waltham, USA). The inhibition percentages (IP) were calculated with the equation:

$$IP = \left(\frac{ABS_{control} - (ABS_{extract} - ABS_{blank})}{ABS_{control}} \right) \times 100$$

Where $ABS_{control}$, $ABS_{extract}$, and ABS_{blank} correspond to the absorbance of the negative control, inhibitor (LME or acarbose), and PB, respectively.

Statistical analyses

The total C and N content of leaves, and the quantification of P, Na, K, Ca, and Mg, antioxidant capacity, and protein content were expressed in mean $\pm$ standard deviation (in percentage, or cmol/kg). The total content of alkaloids, terpenes, phenols, and flavonoids was expressed in mean $\pm$ standard deviation to the chemical equivalents of berberine, gallic acid, quercetin, or dry weight. Quantification of individual phenolic compounds is expressed as mean $\pm$ standard deviation (in $\mu\text{g/g}$ of sample). The α -glucosidase enzymatic inhibition percentages were transformed to the square root of the arcsine and analyzed using a t-test. Statistical analyses were performed with the Agricolae library (De Mendiburu, 2010) in R software v. 4.1.2 (R Core Team, 2020).

Results

Elemental analyses: total carbon, nitrogen, and mineral content

The leaves of *P. padifolia* exhibited high levels of C ($44 \pm 1.15\%$), Na (56.04 ± 1.05 cmol/kg), Ca (74.1 ± 1.65 cmol/kg), and Mg (54.9 ± 1.83 cmol/kg), while displaying low levels of N ($2.31 \pm 0.38\%$), P (4.0 ± 0.03 cmol/kg), and K (2.08 ± 0.08 cmol/kg) (Table 1).

Total content of alkaloids, phenols, flavonoids, terpenes, and proteins

The concentration of secondary metabolites in *P. padifolia* showed variations, with the highest concentration of alkaloids (4.9 ± 0.30 mg/g chemical equivalents of berberine), phenols (3.32 ± 2.47 mg/g chemical equivalents of gallic acid), and terpenes (3.0 ± 0.67 mg/g dry weight) being present at high levels, while flavonoids (0.59 ± 0.15 mg/g

Table 1: Elemental analysis of *Palicourea padifolia* (Humb. & Bonpl. ex Roem. & Schult.) C.M. Taylor & Lorence; values of C, N ratio (%) and content of leaf elements (cmol/Kg) present in leaves (average $\pm$ standard deviation).

Element	Content (% or cmol/kg)
C	44 ± 1.15
N	2.31 ± 0.38
P	4.0 ± 0.03
Na	56.04 ± 1.05
K	2.08 ± 0.08
Ca	74.1 ± 1.65
Mg	54.9 ± 1.83

equivalent amounts of quercetin) were present at low levels. *Palicourea padifolia* leaves had a poor total protein content of 0.02 ± 0.0008 mg/g of albumin equivalents (Table 2). This was consistent with the low N content.

Composition and quantification of phenolic compounds by UPLC-ESI-MS-MS

Eleven phenolic compounds were identified in the leaves of *P. padifolia*, categorized into three chemical groups: phenols (seven compounds), phenolic aldehydes (one compound), flavonoids (two compounds), and coumarins (one compound) (Table 3). The most abundant compounds were chlorogenic acid (335.88 $\mu\text{g/g}$), followed by scopoletin (16.22 $\mu\text{g/g}$), trans-cinnamic acid (10.99 $\mu\text{g/g}$), and (-)-epicatechin (7.84 $\mu\text{g/g}$).

Table 2: Total content of alkaloids (mg/g of berberine equivalents), phenols (mg/g of gallic acid equivalents), flavonoids (mg/g of quercetin equivalents), terpenes (mg/g of dry weight), and proteins (mg/g of albumin equivalents) in *Palicourea padifolia* (Humb. & Bonpl. ex Roem. & Schult.) C.M. Taylor & Lorence. The table shows the average of three replicates + standard deviation.

Metabolites	Total content
Alkaloids	4.9 ± 0.30
Phenols	3.32 ± 2.47
Flavonoids	0.59 ± 0.15
Terpenes	3.0 ± 0.67
Proteins	0.02 ± 0.0008



Table 3: Composition and content of phenolic compounds by UPLC-ESI-MS-MS (dMRM) in methanolic extracts of *Palicourea padifolia* (Humb. & Bonpl. ex Roem. & Schult.) C.M. Taylor & Lorence leaves. Data are expressed as mean $\pm$ standard deviation (n=3). *=Values below the limit of quantification.

Compounds	Concentration in $\mu\text{g/g}$ of crude drug
Phenolic acids	
Chlorogenic acid	335.88 $\pm$ 265.17
Trans-cinnamic acid	10.99 $\pm$ 0.77
Vanillic acid	3.89 $\pm$ 1.96
Caffeic acid	2.65 $\pm$ 1.42
Ferulic acid	0.85 $\pm$ 0.47*
4-hydroxybenzoic acid	0.39 $\pm$ 0.24
4-coumaric acid	0.28 $\pm$ 0.26*
Phenolic aldehydes	
Vanillin	0.18 $\pm$ 0.04*
Flavonoids	
(-)-Epicatechin	7.84 $\pm$ 7.91
(+)-Catechin	0.83 $\pm$ 0.31*
Coumarin	
Scopoletin	16.22 $\pm$ 3.58

GC-MS analysis of leaf extract

Twenty-two compounds were identified in the low polarity fraction of the *P. padifolia* extract by GC-MS analysis, which includes fatty acids (6), aliphatic compounds (3), terpenes (3), aldehydes (3), alcohols (3), ketones (1), esters (1), ethers (1), and tocopherols (1). The major compounds identified in the plant extract were nonacosane, α -tocopherol, phytol, palmitic acid, 7-tetradecenal, (Z)-, octadecanoic acid, and linoleic acid (Table 4).

Antioxidant capacity of leaves extracts

The LME of *P. padifolia* inhibited the DPPH radical with 78.19% and the ABTS radical with 1.25 mg of ET/g (Table 5).

In vitro α -glucosidase enzyme inhibition

The leaf extract showed *in vitro* α -glucosidase enzyme inhibition with a value of 7.05% $\pm$ 4.48%, a low efficacy compared to acarbose, the reference drug with an α -glucosidase inhibition of 83.44 $\pm$ 0.44% (t=3.5261, gl=5, P<0.05) (Fig. 2).

Table 4: Constituents (Area %) of *Palicourea padifolia* (Humb. & Bonpl. ex Roem. & Schult.) C.M. Taylor & Lorence present in the leaf extract analyzed by GC-MS. Abbreviations represent retention time in minutes (Rt), relative peak area (RA), and percentage of similarity (% S). RA data shows an average of triplicate samples (mean $\pm$ standard deviation). *=compounds that were corroborated with a reference standard.

Compound	Rt	RA (%)	S (%)
Fatty acids			
Palmitic acid	22.802	15.40 $\pm$ 10.29	94
Octadecanoic acid	24.747	4.67 $\pm$ 2.95	95
Linoleic acid	24.467	3.69 $\pm$ 3.20	97
Palmitic acid, ethyl ester	23.145	1.24 $\pm$ 1.09	95
Linoleic acid ethyl ester	24.795	1.04 $\pm$ 0.90	90
Tetradecanoic acid	20.685	0.73 $\pm$ 0.64	93
Aliphatic compounds			
Nonacosane	26.779	1.75 $\pm$ 1.65	82
3-Eicosyne	21.817	1.30 $\pm$ 2.26	92
2-methylhexacosane	23.561	1.01 $\pm$ 0.89	94
Aldehydes			
7-Tetradecenal, (Z)-	24.53	13.06 $\pm$ 3.91	90
7-Hecadecanal, (Z)-	11.12	0.68 $\pm$ 0.075	89
Hexadecanal	21.363	0.38 $\pm$ 0.66	94
Alcohols			
1-Dodecanol	17.351	0.71 $\pm$ 0.61	97
1-Decanol, 2-hexyl-	19.755	0.50 $\pm$ 0.87	87
1-Nonanol, 4,8-dimethyl-	13.498	0.17 $\pm$ 0.29	89
Ketones			
Hexahydropseudoionone	16.323	0.93 $\pm$ 1.60	97
Esters			
Diisooctyl phthalate	24.232	1.86 $\pm$ 1.64	94
Ethers			
Benzyl p-nitrophenyl ether	23.783	0.45 $\pm$ 0.39	88
Tocopherols			
α -Tocopherol	25.839	1.45 $\pm$ 0.09	85
Terpenes			
Phytol	24.282	29.30 $\pm$ 6.73	100*
Phytol acetate	21.564	7.82 $\pm$ 2.76	100*
Hexahydrofarnesyl acetone	21.614	1.30 $\pm$ 2.25	95

Table 5: Inhibition of DPPH and ABTS radicals of *Palicourea padifolia* (Humb. & Bonpl. ex Roem. & Schult.) C.M. Taylor & Lorence leaf extracts.

Test	Mean $\pm$ SD
DPPH inhibition (%)	78.19 $\pm$ 0.41
ABTS inhibition (mg EQ Trolox/g)	1.25 $\pm$ 0.001



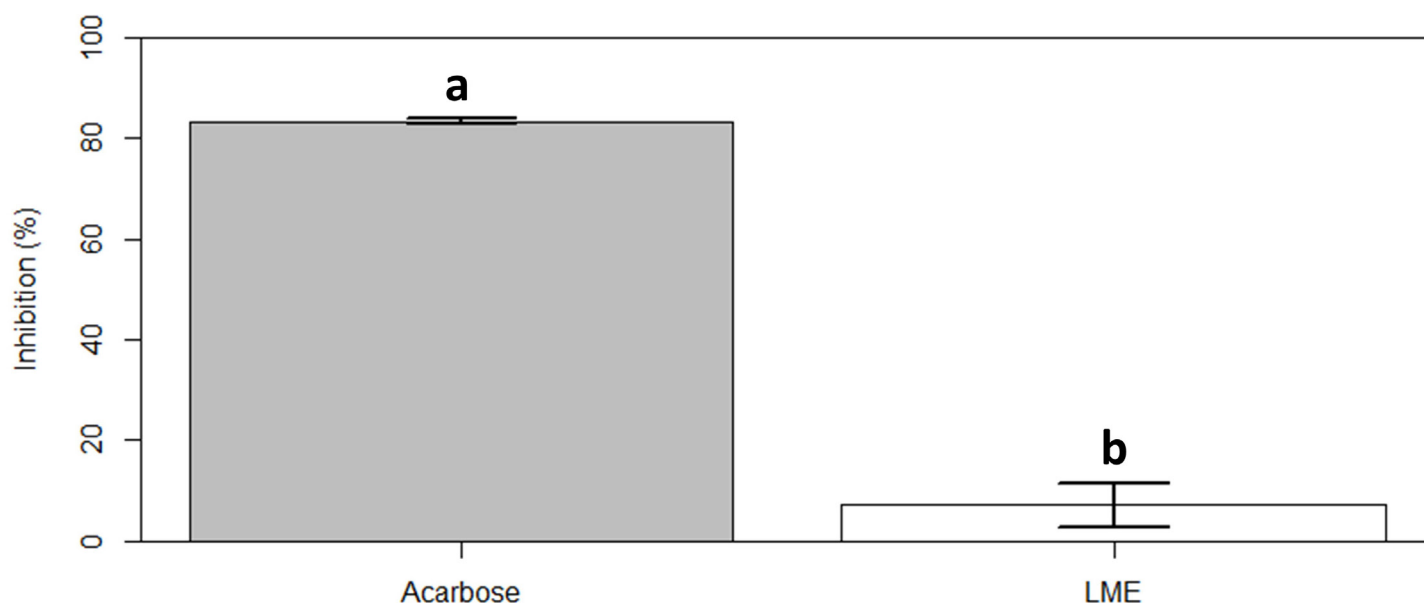


Figure 2: Inhibitory activity of the α -glucosidase enzyme by acarbose and leaf methanolic extract (LME) of *Palicourea padifolia* (Humb. & Bonpl. ex Roem. & Schult.) C.M. Taylor & Lorence (1 mg/ml). Bars represent mean $\pm$ SD of inhibitory activity (3 mM for α -glucosidase), t-test ($P < 0.05$).

Discussion

Elemental analysis of *Palicourea padifolia* leaves

In the present study, *P. padifolia* leaves exhibited elevated levels of C (44%), Ca (74.1 cmol/kg), Na (56.4 cmol/kg), and Mg (54.9 cmol/kg). However, the leaf analysis also showed low levels of N (2.31%), P (4.0 cmol/kg), and K (2.08 cmol/kg) on the tested samples. Although there is no research comparing the nutrient content or macroelements in this species, prior studies indicated that young leaves of *P. rigida* contained high concentrations of K (1.95% (concentration, % dry matter⁻¹)), Ca (0.71%), Mg (0.27%) and P (0.21%) (Amaury de Medeiros and Haridasan, 1985). For mature leaves in the same species, the concentrations reported were K (0.87%), Ca (0.81%), Mg (0.28%), and P (0.05%) (Amaury de Medeiros and Haridasan, 1985). All these values reflect the percentage of elements concerning the dry matter of the sample, respectively. However, nutrients and mineral content of leaves may be influenced by biotic or abiotic factors such as soil type, pH changes induced by the rhizosphere, soil nutrient deficiencies, and abiotic stress (Ishfaq et al., 2022). We suggest that these factors can interfere with the uptake of soil minerals, and they may account for the variation observed in both

studies. Also, in leaves of *Palicourea longiflora* (Aubl.) A. Rich. and *P. rigida*, calcium oxalate crystals in the form of raphides are abundant (Coelho et al., 2007; Tresmondi et al., 2015). The presence of these structures in the leaves of *P. padifolia* could explain the elevated Ca levels obtained in the current study. Although raphides are characteristic structures present in the leaves of species belonging to the genera *Palicourea* and *Psychotria* L. (Tresmondi et al., 2015), it should be evaluated whether they are also present in the leaves of *P. padifolia*.

Total content of alkaloids, phenols, flavonoids, terpenes, and proteins

The Rubiaceae family has diverse compounds including iridoids, anthraquinones, terpenes, flavonoids, and other phenolic derivatives, and alkaloids with special emphasis on bioactive indole alkaloid production (Martins and Nunez, 2015). The LME of *P. padifolia* showed a low concentration of total flavonoids (0.59 mg/g), but a high content of total alkaloids (4.9 mg/g), phenols (3.32 mg/g), and terpenes (3.0 mg/g).

Previous studies have shown that *Palicourea* species such as *P. rigida* have flavonoids and phenols in their leaves



with a total flavonoid content of 3.95 g/100g, and a total phenolic content of 5.78 g/100g (Moraes et al., 2017). Terpene derivatives have also been found in *P. tomentosa* leaves (Formagio et al., 2022).

Plants belonging to the Psycotrieae tribe were shown to be the main producers of diverse alkaloid compounds with considerable structural diversity (Berger et al., 2015; Formagio et al., 2022), as all phytochemical studies with genera belonging to this tribe, such as *Camptotheca* Decne., *Carapichea* Aubl., *Cephaelis* Sw., *Chassalia* Comm. ex Poir., *Margaritopsis* C. Wright, *Psychotria* and *Palicourea* resulted in the isolation of diverse or novel alkaloids (Berger et al., 2015; Martins and Nunes, 2015; Formagio et al., 2022).

In *Palicourea coriacea* (Cham.) K. Schum., alkaloids from the strictosidine pathway were found, including β -carboline-lyaloside (Valverde et al., 1999) and strictosidinic acid. A tetrahydroalkaloid trisaccharide, β -carboline, has also been reported in this species (Do Nascimento et al., 2006). New loganine and secologanine pathway alkaloids were isolated in *P. crocea* (Berger et al., 2015). Considering that alkaloids were also major compounds in the MLE of *P. padifolia*, we suggest expanding bioprospecting studies toward other biological activities. For example, some alkaloids of *Palicourea* spp., especially from the isoquinoline group, are of great interest because of their anti-inflammatory activity. Plant alkaloids also have a wide range of biological activities such as antiplasmodial, cytotoxicity, analgesic, antiviral, and modulators of the activity of the central nervous system (Ohashi et al., 2021).

Determining total protein content is a crucial step in evaluating the nutritional value of plants (Marks et al., 1985). Various studies have analyzed protein sources from edible plants. For example, it has been found that *Psidium guajava* L. shows 98.51 mg of Bovine Serum Albumin (BSA) equivalent/g of Fresh Weight (FW), *Dillenia indica* L. has 13.73 mg BSAE/g of FW, and *Justicia adhatoda* Mart. ex Nees shows 86.37 mg BSAE/g of FW (Sarkar et al., 2020). However, our results indicated that *P. padifolia* leaves have a poor total protein content of 0.02 ± 0.0008 mg/g of albumin equivalents, which is consistent with the low N/C ratio. Nonetheless, this study is the first report about the total leaf protein content in *P. padifolia*.

Phenolic compounds of leaf methanol extracts

Studies on phenolic content in some *Palicourea* species suggest the presence of yet unidentified compounds (Matsuura and Fett-Neto, 2013). For example, flavonol glycosides based on quercetin and kaempferol were identified (Berger et al., 2016). Compounds such as kaempferol 3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside were isolated from *Palicourea crocea*, *Palicourea padifolia* and *Palicourea mortoniana* (Standl.) Borhidi (Berger et al., 2016). Also, benzoic acids are frequently ignored from leaves of the genera *Notopleura* (Hook. f.) Bremek., *Palicourea* and *Psychotria* (Berger et al., 2016).

A low number of chemical compounds were reported in the leaves of *P. padifolia*, and this species had few quantitative determinations. In this sense, our study reported for the first time the presence of eleven compounds belonging to the groups of phenolic acids, phenolic aldehydes, flavonoids, and coumarins, contributing to the phytochemical knowledge of this species. The main compounds found were chlorogenic acid, scopoletin, transcinamic acid, and (-)-epicatechin.

These compounds exhibit a wide range of biological activities; for instance, *Palicourea* and *Psychotria* have been reported to contain chlorogenic acid (Berger et al., 2016). Chlorogenic acid occurs widely throughout the plant kingdom, with high concentrations in apples, peaches, nightshades, and coffee beans (Tundis et al., 2010). This compound has been reported to possess antidiabetic properties (Tundis et al., 2010), as well as antioxidant, neuroprotective, antibacterial, and antiviral properties (Zuo et al., 2015).

Scopoletin, a phenolic coumarin belonging to the phytoalexin group, possesses antioxidant properties, antibacterial activity, and antifungal properties (Gnonlonfin et al., 2012; Lee et al., 2013; Ramírez-Reyes et al., 2019). The use of scopoletin-rich plants in traditional medicine has been known to alleviate conditions such as seizures, inflammation, rheumatic pain, and leprosy (Xia et al., 2007). Scopoletin has been found in the leaves and roots of *Palicourea rigida* (Alves et al., 2017), and in the aerial parts of *Palicourea sessilis* (Vell.) C.M. Taylor (Klein-Júnior et al., 2017; Pinto et al., 2021) and *Psychotria vellosiana* Benth. (Moreno et al.,



2014). Additionally, we here reported scopoletin as a prevalent secondary metabolite of leaves of *P. padifolia*.

Trans-cinnamic acid is a hydroxycinnamic acid present in plants that have shown antibacterial properties (Ramírez-Reyes et al., 2019). In general, hydroxycinnamic acids are compounds with antioxidant properties and their participation in the prevention of stomach cancer has been described (Ferguson et al., 2005). Cinnamic acid is commonly used as a flavoring in foods and beverages, and due to its aroma, it is used in perfumes and cosmetics (Poklar Ulrih et al., 2021).

Epicatechin is a flavonoid with antioxidant, anti-inflammatory, and diuretic properties (Mariano et al., 2018). Consumption of epicatechin has been found to lower blood glucose levels in diabetic patients and to have anti-cancer effects, which are attributed to its antioxidant power and direct cytotoxicity to cancer cells (Abdulkhaleq et al., 2017). Recently, the presence of this compound in aerial parts of *P. minutiflora* (5 mg/g) was reported (De Moura et al., 2020b), which agrees with our findings in the leaves of *P. padifolia*, which showed a concentration of 7.84 ± 7.91 µg/g of crude drug.

Volatile and lipidic compounds found in LPE of leaves

The extract of *P. padifolia* analyzed by GC-MS indicates that the major compounds identified were phytol, palmitic acid, 7-tetradecenal, (Z)-octadecanoic, and linoleic acids. Phytol is commonly found in the leaves of *P. rigida*, and this diterpene has been associated with anti-inflammatory potential in hexane extracts of leaves of this species (Alves et al., 2016) and has antioxidant potential (Islam et al., 2018). Another major compound, palmitic acid, also had anti-inflammatory properties by competitively inhibiting phospholipase A2 (Aparna et al., 2012). (Z)-Octadecanoic acid is a compound that has been found in leaf crude extracts of *P. croceoides* (Udegbum et al., 2017) and has been reported with significant antioxidant potential (Wang et al., 2007). *Premna mucronata* Roxb, a plant rich in 7-tetradecenal, (Z)-octadecanoic acid, phytol, and palmitic acid in its leaves, reveals a potent antioxidant and anti-inflammatory activities (Palariya et al., 2019).

Antioxidant capacity of leaves

Antioxidants are compounds that prevent or reduce the oxidation of molecules, protecting the cells of the human body by inhibiting the formation of free radicals, neutralizing them, converting them into less harmful molecules, and repairing oxidative damage (Kumar et al., 2014). The main results of our study revealed a remarkable antioxidant capacity of leaves of *P. padifolia* that might be closely related to its chemical composition. Chlorogenic acid, trans-cinnamic acids, scopoletin, and epicatechin had potent antioxidant activity reported (Ferguson et al., 2005; Tundis et al., 2010; Mariano et al., 2018). Also, some fatty acids, terpenes, and tocopherols are strong antioxidants and have an important role in health care (Kumar et al., 2014). *Palicourea padifolia* contains low polar compounds in its leaves, including palmitic acid, palmitic acid ethyl ester, phytol, and α -tocopherol, which are reported to promote antioxidant activity significantly (Tucker and Townsend, 2005; Zayed et al., 2014; Prasath et al., 2020; Ali et al., 2022). Indeed, α -tocopherol is one of the eight isoforms of vitamin E and is the most potent fat-soluble antioxidant known in nature (Tucker and Townsend, 2005). This supports the hypothesis that some polar and low polarity compounds present in *P. padifolia* leaves could be responsible for the high antioxidant capacity observed.

α -Glucosidase enzyme inhibition

Reports indicate that within the Rubiaceae family, approximately 34 species of plants possess antidiabetic properties (Sadino et al., 2018). For example, methanol extracts from *Psychotria dalzellii* Hook.f. have exhibited strong inhibitory activity of α -amylase and α -glucosidase enzymes (Abhishek et al., 2019). The inhibition of α -amylase and α -glucosidase enzymes, which delay carbohydrate digestion, is a critical treatment objective for diseases like type 2 diabetes mellitus (WHO, 2013). Inhibiting these enzymes leads to a decrease in glucose absorption, which is viewed as an antihyperglycemic effect (Bischoff, 1994; Tundis et al., 2010; WHO, 2013). This is the first study to report on the inhibitory effect of *P. padifolia* over the α -glucosidase enzyme. Albeit with low potency, the crude leaf extract of *P. padifolia* exhibited a low *in vitro* inhibition (7.05%) of the



α -glucosidase enzyme. Previous studies have reported that chlorogenic acid and scopoletin have inhibitory properties against the α -glucosidase enzyme (Abdullah et al., 2016). We found these compounds in the LME; however, the leaf extract is a complex mix, and the most abundant components could interact with chlorogenic acid, reducing the α -glucosidase inhibition.

Conclusion

The leaves of *P. padifolia* contain significant quantities of Ca, Na, and Mg. The methanol extract of leaves revealed the presence of alkaloids, phenols, flavonoids, and terpenes. The analysis of phenolic compounds determined the existence of eleven compounds in the leaves of this plant. Chlorogenic acid, scopoletin, trans-cinnamic acid, and (-)-epicatechin were the main components found. GC-MS analyses identified phytol, palmitic acid, 7-tetradecenal, (Z)-octadecanoic, and linoleic acid as major compounds. Leaves of *P. padifolia* demonstrated high antioxidant capacity *in vitro*, a low protein content, and LME showed a low inhibition of the enzyme α -glucosidase. This is the first recorded documentation of these biological activities in this species. Phenols, coumarins, and low polar compounds such as terpenes, fatty acids, and α -tocopherol found in LME and LPE are possibly strongly linked to these observed bioactivities. Further *in vitro* studies must be performed using extracts of different polarity to get more conclusive results.

Author contributions

DAIR, MJAM, CLC, and JAGA contributed to conceptualization, funding, methodology, investigation, formal analysis, and writing the original draft. ALKM and JLMV, performed the acquisition and interpretation of mass spectrometry data, as well as contributed to the methodology. NJT contributed to the antioxidant methodology. JEVG and SGVM contributed to the nutrient methodology, and also participated in writing-review and editing. All authors contributed to the discussion, review, and approval of the final manuscript.

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