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Chemical constituents of non-polar fractions and biological activities of *Eugenia myrcianthes* (Myrtaceae) leaves

Constituyentes químicos de fracciones no polares y actividades biológicas de las hojas de *Eugenia myrcianthes* (Myrtaceae)

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Abstract

The genus *Eugenia* (Myrtaceae) represents an important source of medicinal species, with documented biological activities attributed to its diverse secondary metabolites, particularly phenolic compounds and terpenoids. However, comprehensive toxicological evaluation remains essential to validate the safety of traditional applications. This research aimed to evaluate the chemical constituents of non-polar fractions and the bioactive potential of *Eugenia myrcianthes* (*Yva hái*) leaves. Analyses were performed on hexane, chloroform, and aqueous fractions obtained from the ethanolic extract of the plant material. Phytochemical screening revealed the presence of phenolic compounds, flavonoids, tannins, saponins, and triterpenes/steroids. Gas chromatography-mass spectrometry (GC-MS) identified major bioactive constituents as δ-cadinene, α-tocopherol, phytol, and squalene in the chloroform fraction, while τ-muurolol, stigmasterol, β-amyrone, and lupenyl acetate were detected in the hexane phase. Bioactivity assessment demonstrated remarkable

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antioxidant capacity in the aqueous portion (DPPH IC_{50} = 18.5±0.33 $\mu g.mL^{-1}$), contrasting with limited antimicrobial effects against *Escherichia coli, Salmonella typhimurium, Staphylococcus epidermidis,* and *Staphylococcus aureus* strains at ≤2500 $\mu g.mL^{-1}$. Furthermore, no toxicity was detected at ≤20 $mg.mL^{-1}$ using *Drosophila melanogaster* as a model organism. These findings highlight *E. myrcianthes* as a promising source of natural antioxidants. Further research into its specific bioactive compounds is necessary to fully assess the plant's therapeutic potential.

Keywords: *Yva hái*; phenolic compounds; terpenes; DPPH; fruit fly.

Resumen

El género *Eugenia* (Myrtaceae) representa una importante fuente de especies medicinales, con actividades biológicas documentadas atribuidas a sus diversos metabolitos secundarios, en particular compuestos fenólicos y terpenoides. Sin embargo, la evaluación toxicológica exhaustiva sigue siendo esencial para validar la seguridad de las aplicaciones tradicionales. El objetivo de este trabajo fue evaluar los componentes químicos de las fracciones no polares y el potencial bioactivo de las hojas de *Eugenia myrcianthes* (*Yva hái*). Se realizaron análisis de las fracciones hexánica, clorofórmica y acuosa obtenidas a partir del extracto etanólico del material vegetal. El cribado fitoquímico reveló la presencia de compuestos fenólicos, flavonoides, taninos, saponinas y triterpenos/esteroides. La cromatografía de gases-espectrometría de masas (GC-MS) identificó los principales componentes bioactivos como δ-cadineno, α-tocoferol, fitol y escualeno en la fracción clorofórmica, mientras que en la fase hexánica se detectaron τ-muurolol, estigmasterol, β-amirona y acetato de lupenilo. La evaluación de la bioactividad demostró una notable capacidad antioxidante en la porción acuosa (DPPH IC_{50} = 18,5±0,33 $\mu g{\cdot}mL^{-1}$), que contrasta con los limitados efectos antimicrobianos contra las cepas de *Escherichia coli, Salmonella typhimurium, Staphylococcus epidermidis, Staphylococcus aureus* strains a ≤2500 $\mu g{\cdot}mL^{-1}$. Además, no se detectó toxicidad a ≤20 $mg{\cdot}mL^{-1}$ utilizando *Drosophila melanogaster* como organismo modelo. Estos hallazgos destacan a *E. myrcianthes* como una fuente prometedora de antioxidantes naturales. Es necesario continuar con la investigación de sus compuestos bioactivos específicos para evaluar plenamente el potencial terapéutico de la planta.

Palabras clave: *Yva hái*; compuestos fenólicos; terpenos; DPPH; mosca de la fruta.

INTRODUCTION

Empirical use of herbal medicines has historically contributed to modern pharmacotherapy by identifying bioactive compounds (Najmi *et al*., 2022). While synthetic pharmaceuticals dominate contemporary medicine, traditional plant-based therapies maintain cultural and therapeutic relevance throughout Latin America (Lima *et al*., 2018; WHO, 2002). In Paraguay, this practice is deeply rooted in Guaraní ethnobotanical knowledge that has been preserved over generations, with leaf preparations typically consumed in cold beverages (tereré: a traditional Paraguayan drink) or as infusions (Degen de Arrúa & González, 2014).

Among the pharmacologically important families, Myrtaceae stands out for its economic importance, providing edible fruits, wood, and medicinal applications (Rebollar & Tapia, 2016). The genus *Eugenia*, in particular, is rich in bioactive compounds such as flavonoids, tannins, saponins, and terpenes, which are associated with diverse therapeutic properties, including antidiabetic, anti-inflammatory, antimicrobial, and antioxidant effects (Bastos *et al*., 2019; Peixoto *et al*., 2021; de Souza *et al*., 2018).

E. myrcianthes, or *Yva hái*, is a tree with simple, opposite leaves, white and hairy flowers, and bitter-sweet yellow fruits. The geographical distribution corresponds to Argentina, Bolivia, Brazil, Uruguay, and Paraguay (López *et al*., 2002; Pin *et al*., 2009). Previous studies have revealed significant regional variation in its phytochemical profile and bioactivity. Brazilian specimens have demonstrated particularly high antioxidant capacity in leaf extracts, attributed to phenolic compounds such as gallic acid, myricetin, and quercetin (Infante *et al*., 2016). Conversely, Uruguayan samples have shown notable antimicrobial effects against clinically relevant pathogens, including *Staphylococcus aureus* and *Escherichia coli* (Olivaro, 2015).

Although *E. myrcianthes* is traditionally used in Paraguay, its phytochemical profile and bioactive properties lack comprehensive scientific validation. This is particularly significant given the metabolic diversity within Myrtaceae species, the global demand for novel antimicrobial and antioxidant compounds, and the documented risks associated with herbal preparations (42.9% of plant-derived poisonings; Sánchez *et al*., 2019). Based on these considerations, the present study evaluated the lipophilic profile, antioxidant/antimicrobial activities, and toxicity of hexane, chloroform, and aqueous fractions obtained from ethanolic leaf extracts.

METHODS

Plant collection and identification

Leaves of *Eugenia myrcianthes* Nied., were collected in PARAGUAY, Asunción capital city, at the Botanical and Zoological Garden of Asunción (25° 15' 6.32" S, 57° 34' 2.07" W), on 22-VII-2021, by Letizia Grau (N° LG 001). The taxonomic identification was performed by Lic. Fátima Piris da Motta at the Laboratory of Plant Resource Analysis of the Faculty of Exact and Natural Sciences of the National University of Asunción. Finally, a voucher specimen N° 4980 was deposited in the *Herbario Facen*.

Extraction and fractionation procedures

The leaves of *E. myrcianthes* were dehydrated at room temperature for over 10 days and then manually ground into a fine powder. Subsequently, 500 g of the powdered material was macerated in 96% ethanol for 7 days. The resulting mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated by rotary evaporation to obtain the crude ethanolic extract.

The crude extract was partitioned with hexane, producing a hexane-soluble phase and a residual material. The residue was dissolved in water and further separated by liquid-liquid extraction with chloroform using a separatory funnel, resulting in an aqueous phase and a chloroform-soluble portion. The hexane and chloroform phases were concentrated under reduced pressure, while the aqueous portion was lyophilized to obtain a solid for subsequent analysis (Jones & Kinghorn, 2012).

Preliminary phytochemical screening

Precipitation and complex formation assays were employed for the qualitative phytochemical analysis. Alkaloids were detected using Mayer's, Dragendorff's, and Wagner's reagents, while quinones were identified through the Bornträger reaction. The presence of saponins was determined by the foam test, and triterpenes/sterols were detected using the Liebermann-Burchard reaction (Rondón *et al*., 2018). Phenolic compounds, flavonoids, and coumarins were identified through chemical reactions with ferric chloride, Shinoda's reagent, and a fluorescence assay, respectively (García *et al*., 2019).

Total phenolic content

The Folin-Ciocalteu method was employed for the determination of total phenolic compounds (Campi *et al.*, 2021). Methanolic solutions (1 mg·mL^{-1}) of the fractions were prepared, and the calibration curve was performed using methanol as a blank and methanolic solutions of gallic acid (Sigma-Aldrich, USA) at concentrations ranging from 1 to 16 μg·mL^{-1}, treated similarly to the sample. The results are reported as the mean of triplicate analysis, expressed as milligrams of gallic acid equivalents per gram of fraction ± standard deviation (mg GAE.g^{-1} fraction ± SD).

Gas chromatography-mass spectrometry (GC-MS) analysis

The hexane fraction was initially subjected to column chromatography on silica gel 60 (0.063-0.200 mm) using a 97:3 hexane: ethyl acetate mixture as the mobile phase. Three fractions (hexane fractions 1, 2, and 3) were collected and analyzed by GC-MS.

The fractions were dissolved in HPLC-grade solvents, and 1 μL of each solution was injected in splitless mode into a Shimadzu QP2010 Plus GC-MS system. Helium was the carrier gas with a Supelco SLB-5 MS fused silica column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The detector and interface temperatures were set at 270°C and 250°C, respectively. Mass spectra were acquired in full scan mode (m/z 55-550). The oven temperature program was as follows: initial temperature of 60°C for 4 minutes, followed by a ramp of 6°C/min to 280°C, held for 20 minutes (total run time: 60 minutes).

Compound identification was achieved by comparing the mass spectra of each peak with the NIST08 library using GC-MS Solution Version 2.70 software. Compounds with similarity values > 85% were reported. The Kovats retention index (KI) for each identified compound was calculated based on the retention times of linear alkenes, according to the method described by Kovats (1965).

Antioxidant activity: DDPPH free radical scavenging test

The *in vitro* antioxidant capacity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay (de Torre *et al*., 2019; Xanthopoulou *et al*., 2009). Solutions of DPPH (64 μg·mL^{-1}) and varying concentrations of the aqueous (5-30 μg·mL^{-1}), chloroform (20-100 μg·mL^{-1}), and hexane (10-50 μg·mL^{-1}) fractions were prepared. In a 96-well microplate, 150 μL of each fraction was mixed with 150 μL of DPPH solution, incubated at 37°C for 1 hour, and the absorbance was measured at 492 nm using a microplate reader.

Methanol was used as a blank, and ascorbic acid solutions in methanol (2-10 $\mu g \cdot mL^{-1}$), treated identically to the samples, were used as an antioxidant reference compound (control) for calibration. The half-maximal inhibitory concentration (IC_{50}) value for each fraction was calculated from the linear regression equation ($y = mx + b$) and reported as the mean ± standard deviation of triplicate analyses. ANOVA followed by Tukey's *post-hoc* test ($p < 0.05$) was used to compare the activities of the fractions with the control.

Antimicrobial activity: Minimum Inhibitory Concentration (MIC)

Antimicrobial activity was evaluated using the broth microdilution method in 96-well flat-bottom microtiter plates following Clinical and Laboratory Standards Institute protocols (CLSI, 2008). Test microorganisms were Gram-negative (*Escherichia coli* ATCC WDCM 00013 and *Salmonella typhimurium* ATCC 14028) and Gram-positive strains (*Staphylococcus epidermidis* ATCC 12228 and *Staphylococcus aureus* ATCC 6538). Bacterial suspensions were prepared in 0.9% physiological saline and standardized to 1.5×10^8 $CFU \cdot mL^{-1}$ using the McFarland turbidity scale.

Test fractions were initially dissolved in a solvent mixture containing dimethyl sulfoxide (DMSO), Tween 80, and sterile distilled water to prepare a 5000 $\mu g \cdot mL^{-1}$ stock solution. Two-fold serial dilutions (2500 to 39 $\mu g \cdot mL^{-1}$) were prepared in Mueller-Hinton broth (MHB), alongside antibiotic controls gentamicin, erythromycin, and neomycin (1000 to 31.25 $\mu g \cdot mL^{-1}$). Each well received 10 μL of standardized bacterial suspension, followed by incubation at 35°C for 24 h. Bacterial growth was assessed using 20 μL of 0.01% resazurin solution, with absorbance measured at 620 nm.

Appropriate controls were included: a negative control (inoculum + MHB) and a vehicle control (DMSO + inoculum + MHB). The MIC was determined as the lowest test concentration that inhibited bacterial growth (no resazurin color change). Experiments were performed in triplicate.

Acute toxicity test in *Drosophila melanogaster*

The median lethal dose (LD_{50}) was determined following a modified version of the method described by Graf *et al*. (1984). Standard crosses were established by placing 40 females and 20 males of the *D. melanogaster* Canton S[+] strain. After 72 hours, groups of 100 third-instar larvae were transferred to exposure vessels containing 1.5 g of sterilized mashed potato substrate supplemented with 5 mL of varying concentrations of each fraction (0.625 to 20 $mg \cdot mL^{-1}$), dissolved in 0.1% Tween 80.

Hydrogen peroxide (H_2O_2) at concentrations between 2.04 and 32.64 mg·mL^{-1} was used as a positive control. Following a 7-day exposure period, the number of surviving adult flies was recorded. The LD_{50} values were calculated using Probit analysis according to the method described by Finney (1992).

RESULTS

Phytochemical profile

Qualitative phytochemical screening indicated that extracts from *E. myrcianthes* leaves contain phenolic compounds, flavonoids, tannins, saponins, and steroids/triterpenes. Additionally, quantitative assessment of phenolic content revealed that the aqueous fraction exhibited the highest concentration (Table 1).

Table 1. Phytochemical profile and total phenolic compound content of *E. myrcianthes* leaf fractions.

Tabla 1. Perfil fitoquímico y contenido de compuestos fenólicos totales de las fracciones foliares de *E. myrcianthes*.

Fraction	Phenolic Compounds	Flav	Tan	Cou	Quin	Alk	Trit / Ste	Sap	TPC (mg of GAE. g^{-1} of fraction)
Hexane	+	-	-	-	-	-	+	-	164.22 ± 0.006
Chloroform	+	-	-	-	-	-	+	-	79.28 ± 0.008
Aqueous	+	+	+	-	-	-	+	+	254.0 ± 0.002

References: (+) indicates the presence of a phytochemical group based on precipitate formation or color change; (–) indicates its absence (no observable change). Abbreviations: Flav, flavonoids; Tan, tannins; Cou, coumarins; Quin, quinones; Alk, alkaloids; Tri/Ste, triterpenes/steroids; Sap, saponins; TPC, total phenolic compounds. TPC values are expressed as milligrams of gallic acid equivalents per gram of fraction (mg GAE·g-1 fraction) and reported as the mean ± standard deviation (SD) of triplicate analyses.

Referencias: (+) indica la presencia de un grupo fitoquímico sobre la base de la formación de precipitados o el cambio de color; (-) indica su ausencia (ningún cambio observable). Abreviaturas: Flav, flavonoides; Tan, taninos; Cou, cumarinas; Quin, quinonas; Alk, alcaloides; Tri/Ste, triterpenos/esteroides; Sap, saponinas; TPC, compuestos fenólicos totales. Los valores de TPC se expresan como miligramos equivalentes de ácido gálico por gramo de fracción (mg GAE-g-^{1} fracción) y se presentan como la media ± desviación estándar (SD) del análisis por triplicado.

GC-MS Analysis of fractions

Chemical analysis of the hexane fraction revealed the presence of 27 secondary metabolites, primarily classified into terpenoids and phenolic compounds:

Terpenoids (24 compounds): This group included 17 sesquiterpenes and sesquiterpenoids—ledol, caryophyllene oxide, (+)-ledene, alloaromadendrene, bicyclo[4.4.0]dec-1-ene (2-isopropyl-5-methyl-9-methylene-), δ-cadinol, epiglobulol, palustrol, isoaromadendrene epoxide, α-cubebene, γ-gurjunene, spathulenol, varidiflorene, elemol, bulnesol, isoledene, and τ-muurolol—as well as acyclic derivatives such as nerolidyl acetate and farnesyl acetone.

Higher terpenoids were also identified, including the diterpene alcohol phytol and the triterpene squalene, a key precursor in the biosynthesis of cyclic triterpenes (Dewick, 2009). Additionally, two pentacyclic triterpenes, lupenyl acetate and β-amyrone, were detected.

Phenolic Compounds (3 compounds): The phenolic fraction comprised 3,5-di-tert-butylphenol and the tocopherols (α-tocopherol and γ-tocopherol) (Table 2).

A similar but less diverse terpenoid pattern emerged from the chloroform fraction, which contained only 7 identifiable compounds, including 4 sesquiterpenes (germacrene D, α-murolene, δ-cadinene, and viridiflorol) and 3 major compounds (phytol, squalene, and α-tocopherol) (Table 2).

Table 2. Chemical constituents detected in non-polar fractions of *E. myrcianthes* leaves by GC-MS.

Tabla 2. Constituyentes químicos detectados en fracciones no polares de hojas de *E. myrcianthes* por GC-MS.

N°	RT [a]	Constituents	KI [b]	(%) [c]
		Hexane fraction 1		
1	23.387	Ledol	1530	0.08
2	23.632	Caryophyllene oxide	1507	0.06
3	24.198	(+)-Ledene	1419	0.28
4	24.441	Alloaromadendrene	1386	1.62
5	24.726	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	1464	0.57
6	24.941	δ-Cadinol	1580	1.63
7	28.024	Nerolidyl acetate	1754	0.24
8	41.267	Stigmasterol	2879	0.48
9	42.009	Squalene	2914	12.18
10	44.580	β-Amyrone	2869	12.69
11	47.604	Lupenyl acetate	2987	45.39
		Hexane fraction 2		
12	21.907	3,5-di-tert-butylphenol	1555	0.13
13	23.171	Epiglobulol	1530	0.06
14	23.348	Palustrol	1541	0.10
15	23.601	Isoaromadendrene epoxide	1281	0.37
16	24.446	α-Cubebene	1344	0.79
17	28.126	Farnesyl acetone	1754	0.05
18	47.909	α-Tocopherol	3149	24.52
		Hexane fraction 3		
19	23.340	γ-Gurjunene	1461	0.67
20	23.474	Spathulenol	1536	4.57
21	23.849	Varidiflorene	1419	7.21
22	24.052	Elemol	1522	2.26
23	24.395	Bulnesol	1614	3.53
24	24.582	Isoledene	1419	1.10
25	24.751	τ-Muurolol	1580	10.80
26	32.545	Phytol	2045	48.94
27	46.035	γ-Tocopherol	3036	1.97
		Chloroform fraction		
1	21.265	Germacrene D	1515	3.27
2	21.670	α-Muurolene	1440	4.15
3	22.078	δ-Cadinene	1469	9.00
4	24.281	Viridiflorol	1533	3.36
5	32.408	Phytol	2045	16.18
6	41.708	Squalene	2914	14.19
7	47.425	α-Tocopherol	3149	13.88

References: [a] Retention time (minutes); [b] Kovats retention index calculated; [c] Percentage composition.

Referencias: [a] Tiempo de retención (minutos); [b] Índice de retención de Kovats calculado; [c] Composición porcentual.

In contrast, although preliminary qualitative analysis of the aqueous fraction suggested the presence of flavonoids, tannins, and saponins, these compounds were not detected by GC-MS under the experimental conditions employed.

Antioxidant activity

The aqueous fraction exhibited the strongest antioxidant activity, with an IC_{50} value of 18.5 ± 0.33 $\mu g \cdot mL^{-1}$, showing no statistically significant difference compared to the positive control, ascorbic acid (IC_{50} = 10.7 ± 0.87 $\mu g \cdot mL^{-1}$; $p > 0.05$) (Fig. 1). The hexane and chloroform fractions also displayed antioxidant activity, with IC_{50} values of 44.6 ± 1.25 $\mu g \cdot mL^{-1}$ and 103.1 ± 6.41 $\mu g \cdot mL^{-1}$, respectively. Although these values were significantly higher than ascorbic acid ($p < 0.001$), they still indicate notable radical scavenging potential.

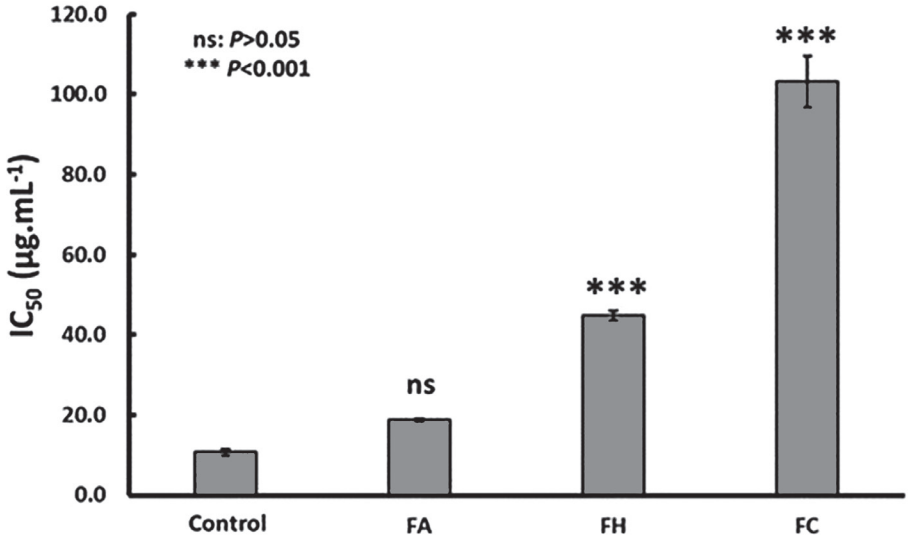

Fig. 1. The IC_{50} values for the radical DPPH scavenging capacity of aqueous (FA), hexane (FH), and chloroform fractions (FC) compared to ascorbic acid (positive control). Lower values indicate higher antioxidant activity. ns: not significant ($p > 0.05$); ***: highly significant difference ($p < 0.001$) relative to the positive control.

Fig. 1. Valores CI_{50} de la capacidad de captación del radical DPPH de las fracciones acuosa (FA), hexánica (FH) y clorofórmica (FC) en comparación con el ácido ascórbico (control positivo). Los valores más bajos indican una mayor actividad antioxidante. ns: no significativo ($p > 0,05$); ***: diferencia altamente significativa ($p < 0,001$) en relación con el control positivo.

Minimum Inhibitory Concentration (MIC)

The fractions derived from *E. myrcianthes* leaves did not exhibit antibacterial activity against the tested strains at any of the concentrations assessed (Table 3). In contrast, the reference antibiotics effectively inhibited bacterial growth, with MIC values of 31.25 $\mu g \cdot mL^{-1}$.

Table 3. MIC values ($\mu g \cdot mL^{-1}$) of *E. myrcianthes* leaf fractions and antibiotics standards against bacterial strains.

Tabla 3. Valores CIM ($\mu g \cdot mL^{-1}$) de las fracciones de las hojas de *E. myrcianthes* y de los antibióticos frente a cepas bacterianas.

Microorganism	Minimum Inhibitory Concentration ($\mu g \cdot mL^{-1}$)					
	FA	FC	FH	Neo	Gen	Ery
E. coli	> 2500	> 2500	> 2500	31.25	31.25	NA
S. typhimurium	> 2500	> 2500	> 2500	31.25	31.25	NA
S. epidermidis	> 2500	> 2500	> 2500	31.25	NA	31.25
S. aureus	> 2500	> 2500	> 2500	31.25	NA	31.25

References: NA: not applicable, FA: aqueous fraction, FH: hexane fraction, FC: chloroform fraction, Neo: neomycin, Gen: gentamicin, and Ery: erythromycin.

Referencias: NA: no aplicable, FA: fracción acuosa, FH: fracción de hexano, FC: fracción de cloroformo, Neo: neomicina, Gen: gentamicina y Ery: eritromicina.

Median lethal dose (LD_{50}) in *Drosophila melanogaster*

Toxicological analysis revealed that none of the tested fractions of *E. myrcianthes* induced mortality in *Drosophila melanogaster* at the highest concentration evaluated, 20 $mg \cdot mL^{-1}$. In contrast, the positive control, hydrogen peroxide, exhibits an LD_{50} of 4.473 $mg \cdot mL^{-1}$ (Table 4).

Table 4. Median lethal dose (LD_{50}) of *E. myrcianthes* leaf fractions evaluated in *D. melanogaster*.

Tabla 4. Dosis letal media (DL_{50}) de las fracciones foliares de *E. myrcianthes* evaluadas en *D. melanogaster.*

Treatment	Total of individuals	Total number of deaths at 20 $mg \cdot mL^{-1}$	LD_{50} $mg \cdot mL^{-1}$	95% Fiducial CI ($mg \cdot mL^{-1}$)	
				Lower	Upper
H_2O_2	100	100	4.473	2.713	7.377
FA	100	4	>20	-	-
FH	100	44	>20	-	-
FC	100	11	>20	-	-

DISCUSSION

Preliminary phytochemical screening revealed phenolic compounds in all the leaf fractions of *E. myrcianthes*. GC-MS analysis identified tocopherol in hexane and chloroform, and no structure was detected in the aqueous fraction under the conditions tested, likely due to several hydroxyl groups requiring derivatization for volatilization (Infante *et al*., 2016). The presence of phenolic compounds has also been described in the hexane extract leaves of *Eugenia involucrate*, which contained tocopherol (Barzotto *et al*., 2019). Moreover, the main compound of the ethanolic extract of *E. involucrate* leaves was (-)-epicatechin and presented 70.19 mg of GAE.g^{-1} extract (Infante *et al*., 2016), lower than reported in this study.

The aqueous fraction showed the strongest antioxidant activity, consistent with its high phenolic content. This relationship supports the well-documented mechanism by which hydroxyl groups enhance antioxidant effects through proton donation (Peñarrieta *et al*., 2017). This result is consistent with the reports of *Eugenia* species, which are characterized by their antioxidant capacity, this is the case of the ethanolic extract of the leaves of *E. leitonii* (IC_{50} = 112.38 $\mu g \cdot mL^{-1}$) and *E. brasiliensis* (IC_{50} = 140.33 $\mu g \cdot mL^{-1}$), which showed lower activity than the reported in this study using the same method (Bastos *et al*., 2019). The chloroform fraction's antioxidant profile (IC_{50}= 103.1 ± 6.41 $\mu g \cdot mL^{-1}$) may be attributed to squalene, phytol, and α-tocopherol, known for their radical-scavenging properties (Huang *et al*., 2009; de Menezes *et al*., 2013; Jiang *et al*., 2022).

Beyond phenolic compounds, non-polar fractions contained diverse sesquiterpenes, including allo-aromadendrene, ledol, spathulenol, epiglobulol, germacrene D, α-muurolene, and δ-cadinene, consistent with the characteristic volatile profile of Myrtaceae species (Apel *et al*., 2005). Despite reports of antimicrobial activity for some sesquiterpenes (Kovács *et al*., 2022), none of the fractions were active against the microorganisms tested – a phenomenon also reported for *E. umbelliflora* and *E. caryophyllata* extracts (Magina *et al*., 2009; Keskin & Toroglu, 2011). Notably, lupeol acetate and ß-amyrone, reported in the hexane fraction, while inactive against *S. aureus*, *E. coli*, and *S. typhimurium*, have demonstrated topical anti-inflammatory effects (Zambrano *et al*., 2017), suggesting potential anti-inflammatory properties for *E. myrcianthes*.

While sesquiterpene-rich fractions of some *Eugenia* species show marked toxicity, as evidenced by *E. uniflora* essential oil causing 78% mortality in *D. melanogaster* at 30 $\mu g \cdot mL^{-1}$ (de Carvalho *et al*., 2017), the hexane fraction of *E. myrcianthes* exhibited only moderate effects, with 44% mortality at the higher concentration of 20 $mg \cdot mL^{-1}$. This reduced toxicity may be attributed to the low concentration (0.08%) of known toxic compounds like ledol (Greeshma *et al*., 2017).

Notably, the aqueous fraction showed no toxicity at 20 $mg \cdot mL^{-1}$, consistent with the safety of hydroalcoholic extracts in rats (5 $g \cdot kg^{-1}$; Rodriguez *et al*., 1992), suggesting that *E. myrcianthes* polar fractions may offer a favorable safety profile for potential applications, though further research is warranted.

CONCLUSION

This study identified 31 phytoconstituents in non-polar fractions of *E. myrcianthes* leaves, predominantly in the hexane phase. The aqueous fraction exhibited superior antioxidant activity, strongly correlating with its high phenolic content. Although sesquiterpenes with reported antimicrobial properties were detected in non-polar fractions, no significant inhibitory effects were observed against the tested bacterial strains.

Notably, all fractions showed no toxicity in *D. melanogaster* at the tested concentrations.

These findings expand the current phytochemical knowledge of this native Paraguayan species and provide a foundation for investigating its therapeutic potential. Future projections should focus on the isolation, purification, and characterization of the identified compounds, and the analysis of the aqueous fraction's bioactive constituents through LC/MS methods, expanding biological activity screening, and conducting *in vivo* evaluations with mammalian model organisms.

ABBREVIATIONS

DDPH: 2,2-diphenyl-1-picrylhydrazyl radical, DMSO: Dimethyl sulfoxide, *E. myrcianthes*: *Eugenia myrcianthes*, GAE: gallic acid equivalents, GC-MS: Gas chromatography-mass spectroscopy, IC_{50}: Half maximal inhibitory concentration, MHB: Mueller-Hinton broth, MIC: Minimum Inhibitory Concentration, NIST: National Institute of Standards and Technology, NMR = Nuclear Magnetic Resonance, TLC: thin layer chromatography.

SUPPLEMENTARY INFORMATION

Supplementary data of Chromatograms of hexane and chloroform fractions are shown as supplementary figures 1, 2, 3 and 4.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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SUPLEMENTARY FIGURES

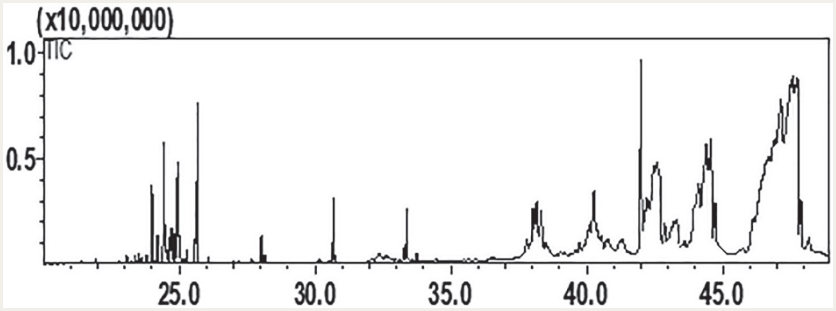

Figure suplementary 1. Chromatogram of Hexane fraction 1.

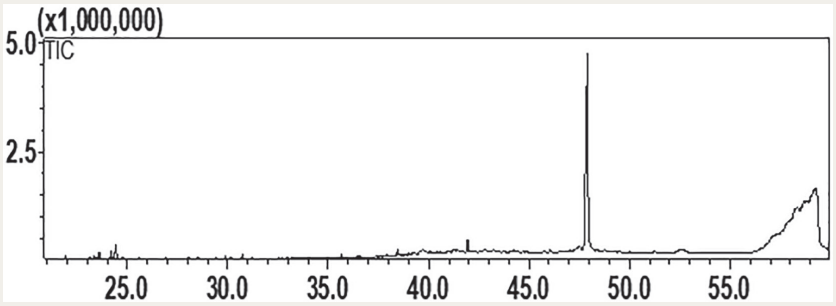

Figure suplementary 2. Chromatogram of Hexane fraction 2.

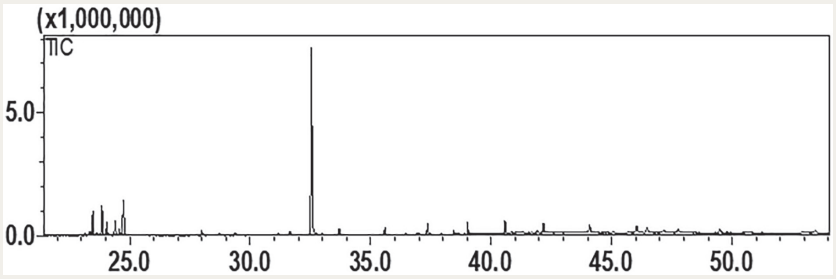

Figure suplementary 3. Chromatogram of Hexane fraction 3.

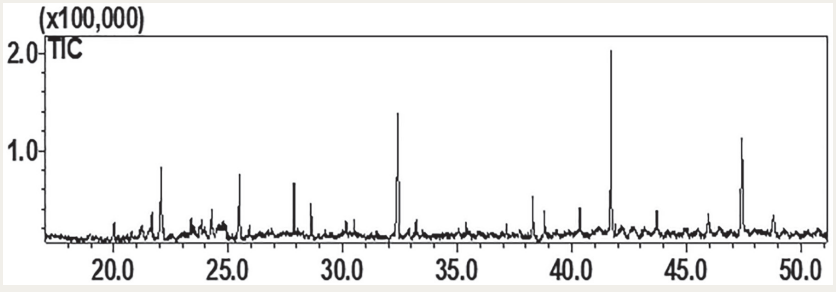

Figure suplementary 4. Chromatogram of Chloroform fraction.