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Chemical composition and antibacterial activity of essential oil *Pluchea carolinensis* (Jacq.) G. Don.(Asteraceae)

Composición química ctividad antibacteriana del aceite esencial de *Pluchea carolinensis* (Jacq.) G. Don. (Asteraceae)

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Abstract

*Currently, plants continue to provide bioactive molecules used as alternative for the development of new drugs. Pluchea carolinensis (Asteraceae) species known as beach sage is used as ornamental plant in some Caribbean locations. Phytochemical studies have reported the presence of sesquiterpenes, lignans, triterpenes and flavonoids. In this regard, the essential oil contains mainly 3-thujopsanone, ß-caryophyllene, spathulenol and ß-chamigrene. Different parts of the plant are used in traditional medicine for the treatment of headache, fiber, stomach conditions, among others. Present study aims to determine the chemical composition of essential oil of P. carolinensis (Jacq.) G. Don collected from Michelena, Tachira State and to evaluate its activity against international reference bacterial strains. Gas chromatography coupled to mass spectrometry assay of the essential oil of the species under study revealed α-pinene (31.94%), thymol (13.60%), bicyclogermacrene (12.64%) and 2,5-dimethoxy-p-cymene (11.10%) as main components. Antibacterial activity assay carried out by agar disk diffusion method showed activity against Staphylococcus aureus ATCC 25923 at minimum inhibitory concentration (**MIC**) of 300 µL/mL and Enterococcus faecalis ATCC 29212 at 500 µL/mL.*

Keywords: *Pluchea carolinensis*, Asteraceae, essential oil, sesquiterpenes, antibacterial activity.

Resumen

*En la actiualidad las plantas continúan proporcionando moléculas bioactivas utilizadas como alternativas para el desarrollo de nuevos fármacos. La especie Pluchea carolinensis (Asteraceae) conocida como salvia de playa es utilizada como planta ornamental en algunas localidades del Caribe. Los estudios fitoquímicos reportan la presencia de sesquiterpenoides, lignanos, triterpenoides y flavonoides. Por su parte, el aceite esencial contiene principalmente 3-tujopsanona, ß-cariofileno, espatulenol y ß-chamigreno. Las diferentes partes de la planta son utilizadas en la medicina tradicional para el tratamiento de dolores de cabeza, fiebres, afecciones estomacales, entre otras. El presente estudio tiene como objetivo determinar la composición química del aceite esencial de la especie P. carolinensis (Jacq.) G. Don. colectada en la población de Michelena en el estado Táchira y evaluar su actividad frente a cepas de referencia internacional. El análisis por cromatografía de gases acoplado a espectrometría de masas del aceite esencial de la especie en estudio reveló como compuestos mayoritarios α-pineno (31,94%), timol (13,60%), biciclogermacreno (12,64%) y 2,5-dimetoxi-p-cimeno (11,10%). Los resultados del ensayo para la actividad antibacteriana realizados por el método de difusión en agar con discos, mostraron actividad frente a Staphylococcus aureus ATCC 25923 a la Concentración Inhibitoria Mínima (**CIM**) de 300 µL/mL y Enterococcus faecalis ATCC 29212 a 500 µL/mL.*

Palabras clave: *Pluchea carolinensis,* Asteraceae, aceite esencial, sesquiterpenos, actividad antibacteriana.

1 Introducción

Increasing bacterial resistance at hospital centers due to incorrect use of antibiotics is considered a worldwide public health problem. In order to counteract the effect of some multi-resistant strains to certain antimicrobial drugs, scientific organizations have the challenge to find new bioactive chemical structures with less secondary effects obtained from natural resources (Buitrago-Díaz et al., 2024; Rojas et al., 2024).

Pluchea Cass (Asteraceae) genus comprises about 80 species distributed in tropical areas of North America, Central America and South America, as well as some species reported in Africa, Asia and Australia (Ibrahim et al., 2022; Gonfa et al., 2022; Hussain et al., 2013; Sharma et al., 2011). Described as aromatic *matojos*, perennial with erect steams, simple leaves, altern and unisexual flowers. In traditional medicine, different parts of the plant, are used for the treatment of headache, fiber, stomach conditions, among others (Elgamal et al., 2021; García et al., 2011).

Pluchea carolinensis (Jacq.) G. Don. known as beach sage is used as ornamental plant in some locations of Cuba, Venezuela and Panamá. Phytochemical studies published about this species indicate the presence of eudesmane type sesquiterpenoids, as well as, lignans, triterpenes and flavonoids. Essential oils are mainly formed by 3-thujopsenone, *ß*-caryophyllene, spathulenol and *ß*-chamigrene (Roersch, 2018; Kerdudo et al., 2016).

Specialized literature stands out the activity of hydroalcoholic extract of *P. carolinensis* leaves against *Enterobacter faecalis*, *Staphylococcus aureus*, *Mycobacterium* sp., *Mycobacterium fortuitum*, *Pseudomonas* sp., *Escherichia coli* and *Klebsiella* sp at concentration values below 100 mg/mL (Pérez et al.; 2007). Another investigation carried out with the ethanolic extract of same species showed antifungal effect against *Candida kefyr*, *Malassezia* sp, *Trichophyton rubrum*, *Trichophyton interdigitale* and *Trichophyton mentagrophytes* with MIC values between 200 to 400 µg/mL (Biabiany et al. 2013).

Presence of flavonoids such as kaempferol, myricetin and quercetin in ethyl acetate and butanol extracts of *P. carolinensis* leaves might be responsible for the high scavenging capacity observed through the *In Vitro* DPPH and ABTS methods at concentrations below to 8.4 mg TE/g of dry extract (Perera et al., 2010).

Antileishmanicidal study with promastigotes of *L. amazonensis* carried out with the hydroalcoholic extract of *P. carolinensis* showed inhibition of 50% at concentration of 30 µg/mL (Garcia et. al., 2011). Compounds like caffeic acid, chlorogenic acid, ferulic acid, quercetin and rosmarinic acid, showed growth inhibition activity of promastigotes and intracellular amastigotes with IC_{50} values between 0.2 and 2.9 µg/mL (Montrieux et al., 2014).

Oral administration, 80 mg/kg, of tincture (30%) obtained from aerial parts of *P. carolinensis* to Wistar rats revealed a reduction of acute and chronic inflammatory process evaluated in two carrageenan-induced rat paw edema and the cotton-induced granuloma model (Rosales et al., 1999).

Present investigation aims to determine the chemical composition of *Pluchea carolinensis* (Jacq.) G. Don. essential oil and to evaluate the growth inhibitory effect on international reference ATCC bacterial strains through the agar diffusion method.

2 Procedimiento Experimental

2.1 Plant material:

Pluchea carolinensis (Jacq.) G. Don. (**Plc**) was collected from Michelena, Táchira state, at 1200 m.a.s.l. (7°56′30″N, 72°14′33″O) in November 2023, during the rainy season and flowering stage. Botanical identification was carried out by Dr. Pablo Meléndez, MERF Herbarium, Faculty of Pharmacy and Bioanalysis, University of Los Andes, Mérida, Venezuela. Voucher specimen was deposited under the following code **JR52**.

2.2 Isolation of essential oils:

Fresh leaves of **Plc** (1100 g) were cut into small pieces and subjected to hydrodistillation for 4 h, using a Clevenger-type apparatus. The oil 0.6 mL (0.05% w/v) was dried over anhydrous sodium sulfate and stored at 4°C until the analyses were performed.

2.3 Gas Chromatography (***GC***)*:*

GC analyses were performed on a Perkin-Elmer AutoSystem gas chromatograph equipped with flame ionization detectors. Two capillary columns of different polarities were used: a 5% phenylmethyl polysiloxane fused-silica column (AT-5, Alltech Associates Inc., Deerfield, IL) (60 m × 0.25 mm, film thickness 0.25 µm) and a polyethylene glycol fused-silica column (AT-WAX, Alltech Associates Inc., Deerfield, IL) of the same dimensions. The initial oven temperature was 60°C; it was then heated to 260°C at 4°C/min and the final temperature was maintained for 20 min. The injector and detector temperatures were 200°C and 250°C, respectively. The carrier gas was helium at 1.0 mL/min and the sample was injected using a split ratio of 1:100. Retention indices were calculated relative to C_8-C_{24} n-alkanes, using only the AT-5 capillary column and comparing values reported in the literature (Adams, 2007; Davies, 1990).

2.4 Gas Chromatography-Mass Spectrometry (***GC-MS***)*:*

GC-MS analyses were carried out on a Hewlett Packard GC-MS system, Model 5973, fitted with a 30 m long, crosslinked 5% phenylmethyl siloxane (HP-5MS, Hewlett Packard, USA) fused-silica column (0.25 mm, film thickness 0.25 µm). The following conditions were applied:

source temperature 230°C; quadrupole temperature 150°C; carrier gas helium, adjusted to a linear velocity of 34 m/s; ionization energy, 70 eV; scan range 40-500 amu; 3.9 scans/s. The injected volume was 1.0 µl of a 2% dilution of oil in n-heptane. A Hewlett-Packard ALS injector was used with a split ratio of 1:100. The identification of the oil components was based on the Wiley Registry of Mass Spectral Data (6th Ed.) and NIST 05 data base library, followed by comparisons of mass spectral (**MS**) data with published literature and the retention index calculation (Adams, 2007).

2.5 Bacterial strains:

The microorganisms used for the antibacterial method were *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 23357) and *Pseudomonas aeruginosa* (ATCC 27853).

2.6 Antibacterial method:

The antibacterial activity was carried out according to the disc diffusion assay described by Velasco et al., (2007). The strains were maintained in agar conservation at room temperature. Each bacterial inoculum was incubated in 2.5 mL Müeller-Hinton broth (BBLTM®) at 37ºC for 18 h (Weng-Alemán et al., 2003). The bacterial inoculum was diluted in sterile 0.85% saline to obtain turbidity visually comparable to McFarland Nº 0.5 standard (1.5 x 10^{6-8} CFU/mL). Every inoculum was spread over plates containing Mueller-Hinton agar and a paper filter disc (6 mm) saturated with 10 µL of essential oil.

The plates were left for 30 min at room temperature and then incubated at 37ºC for 24h. The inhibitory zone around the disc was measured and expressed in mm. A positive control was also assayed to check the sensitivity of the tested organisms using the following antibiotics: Linezolid® (10 µg), Vancomycin® (30 µg), Tobramycin® (30 µg), Aztreonam® (10 µg) and Cefepime® (30 µg). A negative control was also included in the test using a filter paper disc saturated with dimethyl sulphoxide (**DMSO**) to discard any activity of this solvent against the microorganisms assayed. The experiments were repeated twice.

The minimal inhibitory concentration (**MIC**) was determined only with microorganisms that displayed inhibitory zones. **MIC** was determined by dilution of the essential oil in **DMSO** by pipetting 10 µL of each dilution onto a filter paper disc. Dilutions of the oil within a concentration range of 100-600 µL/mL were also carried out. **MIC** was defined as the lowest concentration that inhibited the visible bacterial growth (CLSI, 2024; Buitrago et al., 2023). A negative control was also included in the test using a filter paper disc saturated with **DMSO** to check possible activity of this solvent against the bacteria assayed. The experiments were repeated twice.

3 Results and discussion

Essential oil obtained through hydrodistillation of **Plc** collected from Michelena, Táchira state, was analyzed by by **GC** and **GC-MS**. The chemical profile (Table 1) showed that the oil was composed mainly by sesquiterpenes, where, 25% corresponded to bicyclic sesquiterpenes; 16.67% tricyclic sesquiterpenes; 16.67% oxygenated bicyclic sesquiterpenes and a minor occurrence of other hydrocarbons. Results showed α-pinene (31.94%), thymol (13.60%), bicyclogermacrene (12.64%) and 2,5-dimethoxy-*p*-cymene (11.10%) as major components, while (*E*)-caryophyllene (8.04%), spathulenol (5.00%) and modheph-2-ene (4.77%) showed a moderate presence.

Table 1. Chemical composition of essential oils from *Pluchea carolinensis* (Jacq.) G. Don. leaves.

Compounds	%A	RI
trans-2-hexenal[f]	0.37	846
α-pinene[a]	**31.94**	**932**
thymol[d]	**13.60**	**1289**
modheph-2-ene[c]	4.77	1382
α-isocomene[c]	1.33	1387
(*E*)-caryophyllene[b]	8.04	1417
2,5-dimethoxy-*p*-cymene[d]	**11.10**	**1424**
aristolochene[b]	2.55	1487
byciclogermacrene[b]	**12.64**	**1500**
butylated hydroxytoluene[f]	1.74	1514
spathulenol[e]	5.00	1577
caryophyllene oxide[e]	1.90	1582
[a]Bicylic monoterpene		1 (8.33%)
[b]Bicyclic sesquiterpenes		3 (25%)
[c]Tricyclic sesquiterpenes		2 (16.67%)
[d]Oxygenated monocyclic monoterpene		2 (16.67%)
[e]Oxygenated bicyclic sesquiterpenes		2 (16.67%)
[f]Other hydrocarbons		2 (16.67%)

*Chemical composition was determined by comparison of the MS of each component with Wiley GC/MS library data 6th edition and its retention index (**RI**).*La composición del aceite esencial se determinó por comparación de los EM de cada compuesto con la base de datos Wiley 6ta edición y sus tiempos de retenciòn (**IR**).

Previous investigations on *P. carolinensis* essentials oils of flowers and leaves collected from Cuba revealed through GC-MS analysis the presence 44 constituents representing (F: 64.6%, L: 84.2%) of the total oil analized. The major identified components were selin-11-en-4-α-ol (**F**: 17.7%, **L**: 33.4%), β-caryophyllene (**F**: 5.5%, **L**: 21.1%), 2,5-dimethoxycymene (**F**: 8.9%, **L**: 3.3%), caryophyllene oxide (**F**: 6.6%, **L**: 3.3%), α-pinene (4.7%) and spathulenol (**F**: 3.8%, **L**: 3.1%). Furthermore, two carvotanacetone derivatives were for the first time identified: 5-angeloyloxycarvotagetone and 5-isovaleroyloxycarvotagetone (Kerdudo et al., 2016).

Another investigation conducted with the essential oil of *P. ovalis* aerial parts revealed through **GC-MS** analysis the presence of 37 components, mainly sesquiterpenes. The major chemical components where *α*-cadinol (15.54%), *δ*-cadinene (12.93%), *β*-selinene (5.86%), *τ*-muurolol (4.70%), *β*-gurjurene (4.17%), cubebol (3.00%), *γ*-cadinene (2.99%) and epizonarene (2.69%) (Gonfa et al., 2022).

In another investigation, the essential oil of *P. carolinensis* flowers consisted mainly of aldehydes and esters. The main compound was selin-11-en-4a-ol (43.40%), followed in a smaller proportion by 2,5-dimethoxy-*p*-cymene (12.50%), neryl isovalerate (6.40%) and caryophyllene oxide (6.80%) (Pino et al., 2009). Likewise, the oil from the leaves of *Pluchea pteropoda* collected in Vietnam showed high concentrations of oxygenated monoterpenes and sesquiterpenes, among which 2,5-dimethoxy-*p*-cymene (43.50%), *β*-maaliene (14.00%) and *α*-isocomene (9.00%) were found (Ninh The et al., 2022).

The variation in the chemical composition of the essential oil has been related to biotic and abiotic factors. The type of chemical components biosynthesized by the plant is related to genetic patterns, growth stages, geographic location, soil chemical composition, water availability, seasonal factors, extraction method, storage conditions, among others (Rojas et al., 2023; Madboly et al., 2023; Buitrago et al., 2015). Likewise, some compounds are observed only in specific species, considered as chemotaxonomic markers for species identification and authentication (Rojas et al., 2025).

Antibacterial evaluation assayed in present investigation revealed that **Plc** essential oil was active against grampositive bacteria (Table 2). The oil obtained from the leaves caused growth inhibition of *S. aureus* (**MIC**: 300 µL⁄mL) and *E. faecalis* (**MIC**: 500 µL⁄mL).

Table 2. Antibacterial activity of leaf essential oils of *Pluchea carolinensis* (Jacq.) G. Don.

Microorganisms	Inhibition zone (mm)*						MIC µL/mL
	Essential oil	Antibiotics					
		LI	VA	TO	AZT	CEP	
Staphylococcus aureus (ATCC 25923)	**9***	40*					**300**
Enterococcus faecalis (ATCC 29212)	**8***		26*				**500**
Escherichia coli (ATCC 25922)	NA			34*			NT
Klebsiella pneumoniae (ATCC 23357)	NA				42*		NT
Pseudomonas aeruginosa (ATCC 27853)	NA					38*	NT

*Zone of inhibition (discs 6 mm in diameter), **NA**: not active, **NT**: not tested; **LI**: Linezolid® (10 µg); **VA**: Vancomycin® (30 µg); **TO**: Tobramycin® (30 µg); **AZT**: Aztreonam® (30 µg); **CE**: Cefepime® (30 µg); **MIC**: Minimum inhibitory concentration, Range: 100-600 µL/mL. *Zona de inhibición (discos 6 mm de diámetro). **NA**: no activo; **NP**: no probado; **LI**: Linezolid® (10 µg); **VA**: Vancomicina® (30 µg); **TO**: Tobramicina® (30 µg); **AZT**: Aztreonam® (30 µg); **CE**: Cefepime® (30 µg); **CIM**: Concentración inhibitoria mínima, Rango: 100-600 µL/mL.

According to the classification suggested by Kuete (2010), for natural products evaluation, results obtained in the present investigation show that **Plc** essential oil exhibit moderate (100 µg/mL < **MIC** < 600 µg/mL) activity against *S. aureus* and *E. faecalis* bacteria strains.

Previous investigation on *P. eupatorioides* essential oil demonstrated effectiveness against *Staphylococcus aureus* and *Candida albicans* with **MIC** value of 100 µg/mL. Moreover, the essential oil was also found to be effective against *Bacillus cereus* and *Escherichia coli* with a **MIC** of 200 µg/mL (Thinh et al., 2023).

The essential oil *P. carolinensis* and 3 isolated molecules (5-isovaleroyloxycarvotagetone, 5-angeloyloxycarvotagetone and selin-11-en-4-ol) were significantly active at 0.5 % (w/v) against *A. niger*, *C. albicans*, *S. aureus* and *B. cereus*. No activity was observed against *P. aeruginosa*, *E. coli*, *S. arizonae* and *L. innocua* (Kerdudo et al., 2016).

Another investigation carried out with extracts from fresh leaves of *Pluchea carolinensis* showed growth inhibition of *Klebsiella* sp. at the **MIC** of 1mg/mL. On the other hand, *P. carolinensis* extracts showed activity against *E. faecalis*, *S. aureus*, *Mycobacterium* sp., *M. fortuitum*, *Pseudomonas* sp. and *E. coli* with **MIC** value of 100 mg/mL (Pérez et al., 2007).

The antibacterial activity observed by essential oils could be related mainly to mono- and sesquiterpenes, which have the ability to inhibit bacterial growth through several mechanisms such as: cell membrane denaturation, nutrient transport interference, metabolic regulation, among others (Buitrago et al., 2023; Patra et al., 2016; Trombetta et al., 2005). Some studies have revealed that caryophyllene and caryophyllene oxide have the ability to diffuse the cell membrane causing inhibition of bacterial growth (Sarpietro et al., 2015).

4 Conclusions

The essential oil of *P. carolinensis* leaves is composed mainly by monoterpenes, thymol and *α*-pinene, observed as main components. The antibacterial activity revealed that the essential oil has activity against *S. aureus* and *E. coli* at concentrations of 300 and 500 µL/mL, respectively. According to the results observed, the essential oil of this species may represent an alternative for the treatment of some infectious processes associated with these grampositive bacteria.

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