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COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE *PIPER ERIOPODON* (MIQ.) C.DC. ESSENTIAL OIL FROM THE VENEZUELAN ANDES

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Abstract

The essential oil of the fresh leaves and stems of *Piper eriopodon* (Piperaceae), was extracted by hydro-distillation, using the Clevenger trap, with a yield of 0.188%. Thirty-eight compounds were identified by gas-chromatography, coupled with mass spectrometry (GC-MS). The main components were: 1,8-cineole (37.2%), β-pinene (9.38%), myristicin (7.82%), α-pinene (7.27%), trans-caryophyllene (5.80%) and β-selinene (4.04%). The antibacterial activity assessment was carried out using the disk diffusion agar method technique (Kirby-Bauer). Results showed that the essential oil from the *Piper eriopodon* showed no antibacterial activity against Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*). In contrast, against Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*), it only showed activity against *Staphylococcus aureus*, determining a Minimum Inhibitory Concentration of 2,500 µg/mL.

Keywords: *Piperaceae, Piper eriopodon, antibacterial activity, essential oil.*

Introduction

Piper eriopodon is a species belonging to the Piperaceae family. It is considered a relatively large family of at least 2000 species. Family members are located within the tropics, finding their highest concentrations and dispersion centers in America Latin and Malaysia [1]. The Piperaceae family has great economic importance, especially for the *Piper nigrum* (black pepper) species used as a spice; nevertheless, various medicinal uses for different species of the genus *Piper* have also been described, including anti-inflammatory, analgesic and treatment for snakebite [2]. Recent studies of extracts and essential oils obtained from different *Piper* species have shown **insecticidal activity** for *P. amalago*, *P. umbellatum*, *P. aduncum*, *P. nudum*, *P. diandrum*, *P. psilorhachis*, *P. hispidum* and *P. sanctum* [3]; **antimicrobial activity** for *P. auritum* Kunth [4], *P. betel* [5], *P. augustifolium* [6], *P. pesaresanum*, *P. aniel-gonzalezii*, *P. glanduligerum*, *P. crassinervium*, *P. umbellatum*, *P. crassinervium* [7], *P. amalago* [8], **antifungal activity** for *P. sentifolium* [2], *P. amalago* [8], *P. aduncum* [9], *P. arthante* [10], *P. hispidum*, *P. bogotense*, *P. marginatum*, *P. bremedeyeri* Jaqc., *P. cf. divaricatum* [11]; **antiparasitic activity** for *P. ariatum* [1], *P. acutifolium*, *P. aduncum*, *P. callosum* [13], *P. melastomoides* [14]; **antioxidant activity** for *P. betel* [5], *P. crassinervium* [15], *P. auritum*, *P. imperiale* [16]; **repellent properties** for *P. dilatatum*, *P. divaricatum*, *Piper* sp, *P. santifelicis*, *P. aducum* [17]; and **biocide properties** for *P. elongatum* [18].

Phytochemistry of *Piper* species, especially that of the *P. aduncum*, has been extensively investigated and a large number of physiologically active compounds, such as alkaloids, amides, propenylphenols, lignans, neolignans, terpenes, steroids, flavones, among others, have been identified [19]. Terpenes and triterpenes, saponins, phenols, and tannins were detected from the dichloromethane extracts of the aerial parts of *P. eriopodon* [7]. From the ethanol extract of the wooden parts of *P. eriopodon*, the presence of steroid and/or triterpenoid-like compounds, naphtha and/or anthraquinones and coumarins were detected. There was also possible to extract and identify steroid-type compounds using instrumental-analysis techniques (3- keto, 4-methyl, 22-stigmastene) and a mixture of fatty acids [20].

Studies of both the essential oil and extracts of *P. eriopodon* attribute to it antifungal [15, 19, 21], antioxidant [14,20], antiproliferative [22], cytotoxic [11,23] and herbicide activity [24]; while, no antimicrobial activity has been reported against strains of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomona aeruginosa* [7].

In Venezuela, one species with the greatest geographical distribution is *P. aduncum*, with other species such as *P. dilatatum*, *P. tuberculatum*, *P. eriopodon* and *P. hispidum*. However, studies on the chemical composition of essential oils and extracts of *P. eriopodon* are rare and correspond to the Colombian Piperaceae family [22, 25]. There are no known reports of the chemical composition of this species essential oil in Venezuelan territory. Characteristics of climatic conditions, soil type, height, light intensity, and seasonality create significant differences in the chemical composition of the essential oils of a species [26-29]; highlighting the relevance of studying and characterizing plants of interest according to the geographical area of origin.

This study reports the chemical composition of the essential oil, in fresh leaves and stems, of the *P. eriopodon* species from the Venezuelan Andes and its antibacterial activity on *Staphylococcus aureus*, *Enterococcus fecalis*, *Escherichia coli* and *Klebsiella pneumoniae*.

Methods

Plant material:

Fresh leaves and stems of *P. eriopodon* were collected at the "El Joque" Experimental Station of the University of Los Andes, located in the Parish Jají, Campo Elías Municipality in the State of Mérida, Venezuela (8°34´30"N 71°19´43,4"O) at an altitude ranging from 1,900 to 2,100 m.a.s.l (Figure 1). The average annual temperature and precipitation are 16°C and 1,300 mm of water, respectively, with a relative humidity of 82.2% and an irregular topography with acidic soils. The plant was botanically identified by Engineer Juan Carmona Arzola at the MERF herbarium "Luis Ruiz Terán" of the Faculty of Pharmacy and Bioanalysis of the University of Los Andes.

Extraction of essential oil:

Fresh leaves and stems (800 g) of *P. eriopodon* were liquefied and subjected to distillation by dragging with water vapor (4 hours), using a Clevenger trap. 2 mL of yellow essential oil were extracted (0.188% yield). The oil was kept at -4 °C until it was used for biological tests [30].

Chemical composition analysis:
Gas-chromatography coupled with mass spectrometry (GC-MS)

Volatile components of *P. eriopodon* essential oil were analyzed in a Hewlett Packard Model GC System HP6890 and Mass Selective detector 5973 series II at 70 eV, equipped with an automatic injector, using an HP-5MS capillary column of 30 m of length x 0.25 mm diameter x 0.25 µm film thickness. The temperature program used was initially set at 60°C, then heated at a rate of 4°C per min to 260°C. 1.0 µL of a 2% solution of the essential oil was injected into n-heptane with a 100:1 partition. The injection temperature was 200°C. The identification of the oil components was performed by computerized comparison of the spectra obtained from each compound with the spectra from the Wiley Library (6th Edition). Additionally, the Kováts indexes were determined by comparing each compound's index in the sample with the indexes corresponding to a mixture of n-alkanes (C8 to C22) [31, 32].

Antibacterial activity:

Antibacterial activity was evaluated according to the paper-disk-diffusion agar method [33], using *Staphylococcus aureus* ATCC 25923, *Enterococcus fecalis* ATCC 19433, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 23357 as reference strains provided by the Department of Microbiology and Parasitology of the Faculty of Pharmacy and Bioanalysis of the Universidad de Los Andes.

The activity of *P. eriopodon* essential oil and the determination of the minimum inhibitory concentration (MIC) were carried out from the pure oil and dilutions of the essential oil with dimethyl sulfoxide (DMSO) at concentrations of 5000 µg/mL, 2500 µg/mL and 1250 µg/mL. As a positive control, reference antibiotics Erythromycin® (15 µg), Ampicillin® (10 µg), Amikacina® (30 µg), Piperacillin® (100 µg) were used; and DMSO as a negative control. All tests were performed in duplicate.

Results

Chemical composition of the essential oil:

From the hydro-distillation process, 1.5 mL of essential oil were obtained from the fresh leaves and stems of *P. eriopodon*, which corresponds to a yield of 0.188%. It was analyzed by GC-MS and thirty-six compounds, which represented 96.46% of the mixture, were identified by comparison with the Wiley, Adams, Nist and retention indexes (KI) databases. Table 1 shows the identified volatile compounds of the essential oil from *P. eriopodon*. The results and their analysis show that the components correspond to monoterpenic hydrocarbons, hydroxylated monoterpenes, ester monoterpenes, ether monoterpenes, non-terpenic ether and sesquisterpenes. However, the mixture contains mostly oxygenated monoterpenes (42.38%) and hydrocarbon monoterpenes (25.67%); being 1,8-cineole (37.2%), β-pinene (9.38%), myristicin (7.82%), α-pinene (7.27%), trans-caryophyllene (5.80%)) and β-selinene (4.04%) the most abundant components.

Antibacterial activity:

The essential oil *P. eriopodon* showed antibacterial activity only against *Staphylococcus aureus*. This was determined by the disk-diffusion agar method and represented a MIC of 2500 µg/mL. However, it did not show any activity against *Enterococcus faecalis* or against Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*.

Discussion

These results do not coincide with any studies reported for *P. eriopodon* in Colombia. There were also discrepancies in the composition of essential oils between both published studies in Colombia, probably due to the difference between the variety

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of *P. eriopodon* used. In the botanical samples collected in the departments of Santander, Meta, Bolívar, Sucre, and Cesar, main components were determined as follow: dill apiol (38.8%), trans-β-caryophyllene (8.1%), γ-cadinene + myristicin + 10-epi-cubebol (5.2%), β-selinene (5.0%) and caryophyllene oxide + globulol (3.8%) (25). Meanwhile, in samples collected in rural and semi-urban areas of the Departments of Santander, Arauca, and Bolívar, the main components found were: α-pinene (18.6%), β-pinene (16.1%) and trans-β -caryophyllene (11.6%) [22]. These inconsistencies in the chemical composition of *P. eriopodon* essential oils may be associated with soil conditions, altitude, and climate, among other variables [26-29] between the geographical areas where botanical samples were grown and collected.

Various biological activities (anti-termite, insecticide, acaricide, antifungal, antibacterial, antioxidant, and anti protozoic) are attributed to the significant amount of 1,8-cineol. This component was found in this study, though it is mostly present in eucalyptus essential oils (one of the main genera of the Myrtaceae family). The 1,8-cineole is the principal component of eucalyptus oil, and its percentage varies depending on the species and the country when the plant is grown. It is worth mentioning that medicinal essential oils are designated in terms of 1,8-cineole content [34]. On the other hand, other notorious compounds found were β-pinene, α-pinene, trans-caryophyllene, and β-selinen, which have antifungal activity. Furthermore, trans-caryophyllene has anti-dermatophytic, cytotoxic, and antiprotozoal activity [11, 35]. Finally, myristicin, whose pharmacological potency is comparable to that of the indomethacin, an anti-inflammatory drug [36]; highlighting the importance of the essential oil of *P. eriopodon* in this study.

The antibacterial-activity results could be attributable to the 1,8-cineol, which, as mentioned before, is a significant compound of the *P. eriopodon* essential oil, having previously demonstrated antimicrobial activity against various microorganisms, including *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* [34]. Nonetheless, the lack of antibacterial activity against *Escherichia Staphylococcus coli*, may be caused to a low concentration of (37.2%). Essential oils of different species of Eucalyptus tend to have concentrations of 1,8-cineole ranging between 50 to 90% [34]. This relatively low concentration of 1,8-cineole in the essential oil of *P. eriopodon* could be limiting the biological activity, hence, explaining the weak antibacterial activity reflected in the value of the maximum-inhibition-zone diameter for *Staphylococcus aureus* (6 mm). The study carried out by Vallejo et al. (2014), from dichloromethane extracts of *P. eriopodon* obtained by leaching method, showed no antimicrobial activity against Gram-negative bacilli such as *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomona aeruginosa*, which is consistent with those results obtained from the essential oil of the same botanical species.

Studies based on various species of Eucalyptus, generally, indicate that Gram-positive bacterial strains are more sensitive to essential oils than Gram-negative ones [37-39]. This trend can be rationalized taking into account that Gram-negative bacilli have a lipopolysaccharide membrane that is restrictive for the diffusion of hydrophobic compounds present mainly in essential oils; thus, preventing components from exerting their effect, such as increasing ion permeability, leakage of vital intracellular components, or compromising bacterial enzymes [38,39]. However, other species in the Piperaceae family have shown antibacterial activity against Gram-negative bacilli strains [7].

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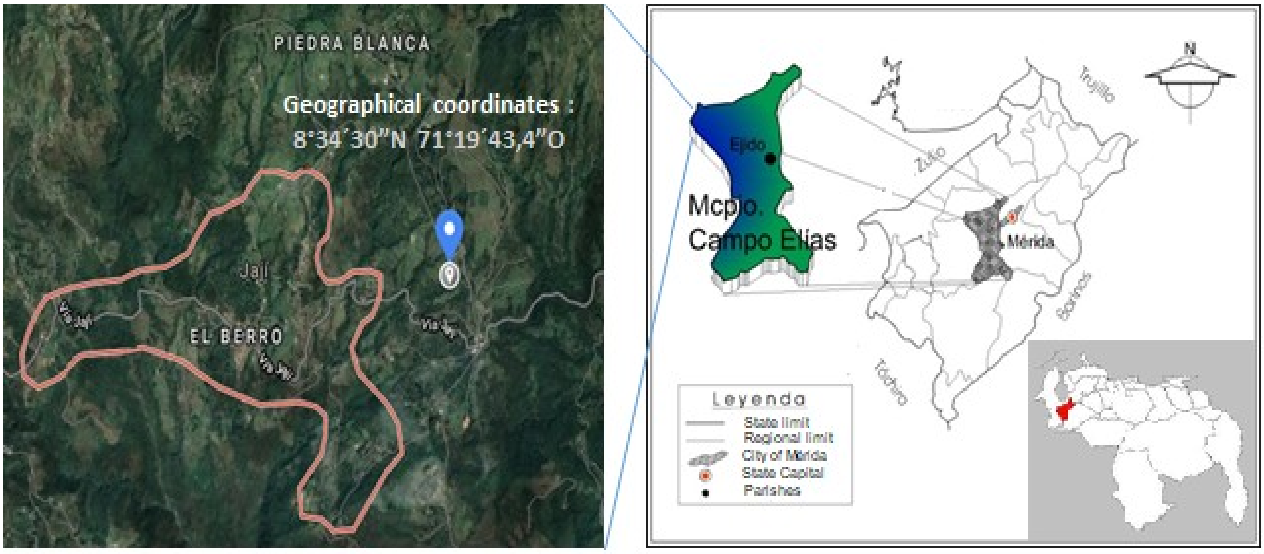

Figure 1. "El Joque" Experimental Station of the University of Los Andes, located in the Parish Jají, Campo Elías Municipality in the State of Mérida, Venezuela.

Table 1 : Identified components of *Piper eriopodon* essential oil

# PK	COMPOUND NAME	TR	%	KI ref	KI calc
1	*trans*- 2-hexenal	3.67	0.17	850	846
2	*E* -3-hexenyl butanoate	3.70	0.13	852	860
3	α - Tujeno	4.97	0.13	931	924
4	**α- pinene**	**5.14**	**7.27**	**939**	**932**
5	Camphene	5.47	0.15	954	946
6	**β- pinene**	**6.11**	**9.38**	**981**	**974**
7	β - myrcene	6.38	2.35	992	988
8	α - felandrene	6.76	0.42	1007	1002
9	α - terpinene	7.08	0.28	1020	1014
10	*m* - cimene	7.30	0.45	1029	1023
11	**1.8 cineole**	**7.56**	**37.2**	**1039**	**1026**
12	*c is* -ocimen	7.62	2.27	1041	1032
13	*trans* - β - ocimeno	7.90	2.18	1052	1044
14	γ- terpinene	8.23	0.53	1064	1054
15	α - terpinolene	9.09	0.26	1092	1086
16	Linalool	9.42	0.11	1103	1095
17	δ- terpineol	11.53	0.78	1171	1162
18	Terpinene-4-ol	11.86	0.56	1180	1174
19	α - terpineol	12.31	3.33	1193	1186
20	*cis* -piperitol	12.45	0.15	1196	1195
21	*t rans* -piperitol	12.83	0.25	1209	1207
22	α - copaeno	18.22	0.54	1378	1374
23	α - gurjuneno	19.28	0.12	1409	1409
24	***trans* - caryophyllene**	**19.61**	**5.80**	**1421**	**1419**
25	(+) - Aromadendrene	20.18	0.57	1442	1439
26	α - humulene	20.64	2.30	1458	1452
27	**β - selinene**	**20.94**	**4.04**	**1468**	**1490**
28	*t rans* -Muurola-4 (14), 5-diene	21.47	1.22	1486	1493
29	β - selinene	21.63	0.79	1492	1489
30	α - selinene	21.90	0.74	1501	1498
31	Pentadecane	21.96	1.76	1503	1500
32	7-epi- α - selinene	22.56	0.11	1522	1520
33	**Myristicin**	**22.72**	**7.82**	**1527**	**1517**
34	Spatulenol	24.31	0.12	1576	1577
35	Caryophylene oxide	24.48	0.67	1581	1582
36	Dill apiol	25.63	1.51	1622	1620

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Total identified compounds	96.46
Hydrocarbon monoterpenes	25.67
Oxygenated monoterpenes	42.38
Hydrocarbon Sesquiterpenes	16.23
Oxygenated Sesquiterpenes	0.79
Phenyl propane derivatives	9.33
Hydrocarbons	2.06

TR: Retention time of the components. %: relative area. KIref: Kováts index extracted from Adams, 2007. KIcalc: Kováts index calculated with the programmed temperature in the HP-5 MS column. (*) Identified by direct comparison with an authentic sample

Table 2: Antibacterial activity of the essential oil of *Piper eriopodon* (Miq.) C. DC

Microorganisms	***Piper eriopodon* essential oil**								
	Average Inhibition Halos (mm) *								
	Concentrations µg / mL								
					Controls (+)				**Control (-)**
	Pure oil	**5000**	**2500**	**1250**	**AN**	**ERI**	**A.M**	**PRL**	**DMSO**
S. aureus ATCC 25923	6 *	6 *	5 *	0 *		30 *			0 *
E. faecalis ATCC 19433	NA						32 *		0 *
E. coli ATCC 25922	NA				30 *				0 *
K. pneumoniae ATCC 23357	NA				28 *				0

Legend: AN: amikacin , AM: ampicillin, PRL: piperacillin , ERI: erythromycin , DMSO: dimethyl sulfoxide , NA : not active