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Chemical composition and antibacterial activity of *Piper lenticellosum* C.D.C essential oil collected in Ecuador

María Eugenia Rondón[1*], Judith Velasco[2], Xavier Cornejo[3], Janeth Fernández[2], Vladimir Morocho[3]

[1]Facultad de Ciencias Naturales, Universidad de Guayaquil, Guayaquil, Ecuador. Facultad de Farmacia y Bioanálisis. Universidad de Los Andes, Mérida, Venezuela. [2]Facultad de Farmacia y Bioanálisis. Universidad de Los Andes, Mérida, Venezuela. [3]Herbario GUAY, Universidad de Guayaquil, Facultad de Ciencias Naturales, Guayaquil, Ecuador. [4]Departamento de Química. Universidad Técnica Particular de Loja, Ecuador.

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ABSTRACT

The essential oil of the leaves and spikes of *Piper lenticellosum* C. DC. (Piperaceae) was isolated by hydrodistillation and analyzed by GC/MS. A yield of 2.06 % was obtained. Twenty eight components were identified by comparison of their mass spectra with Wiley GC-MS library data and the retention indices (RI) calculated for every compound. The major constituents were piperitone (33.97 %), 1,8 cineole (11.92 %), limonene (11.07 %), safrole (8.18 %) and α-pinene (4.49 %). Antibacterial activity of the essential oil was evaluated against five important human pathogenic bacterial strains using the disk diffusion agar method. The results showed at moderate activity against *Staphylococcus aureus* ATCC (25923), *Escherichia coli* ATCC (25922) and *Klebsiella pneumoniae* ATCC (233357) with minimal inhibitory concentration (MIC) values of 100, 200 y 300 µL/mL, respectively. According to the literature consulted, this is the first report on chemical composition and antibacterial activity of the essential oil of *Piper lenticelosum* C. DC. from Ecuador.

INTRODUCTION

The genus *Piper* L. (Piperaceae) comprises more than 700 species widely distributed in the tropical and subtropical regions of the world (Jaramillo and Manaos, 2001); and is known for its economic importance due at its aromatic and medicinal properties (Ravindran, 2000). Particularly, in Ecuador 275 *Piper* species have been reported, 75 of which are endemic to the country (Callejas, 1999; Quijano *et al*., 2006). Traditionally, *Piper* species have been used in Latin America as analgesics in pain management, toothache and wound treatment (Parmar *et al*., 1997). In Ecuador, some of this species have been used in folk medicine to treat numerous diseases as bronchitis, dysmenorrheal, anti-diarrheal, anti-parasitical, renal diseases and the leaves bath against several indispositions (Grandtner and Chevrette, 2013). The essential oils of *Piper* species have been recognized for showing insecticidal, bactericidal, larvicidal, molluscicidal, cytotoxic, anticholinesterasic and leishmanicidal activities (da Silva et al., 2014; Morales *et al*., 2013). The chemical composition of essential oil of some *Piper* species have been analyzed showing as main compounds phenylpropanoids such as safrole, dillapiol and myristicin and terpenes such as limonene, β-caryophyllene, spathulenol, (*E*)-nerolidol, α-bicyclogermacrene and cadinol (Maia *et al*., 2009; Moura do Carmo *et al*., 2012). *Piper lenticellosum* C. DC., has been cited as a synonym of *P. carpunya* Ruiz & Pav. (Jorgensen & León, 1999), it is known in Ecuador as "guaviduca", and has been used in traditional medicine as anti-inflammatory, anti-ulcer, anti-diarrheal and anti-parasitical remedy as well as a treatment for skin irritations (Diaz and Dorado, 1986).

Despite the wide numbers of studies on chemical composition from *Piper* species, a single report on essential oil of *Piper carpunya* from Peruvian Amazon is available (Vargas et al., 2004). Anti-secretory, anti-inflammatory and anti-*Helicobacter pylori* activities such as antioxidant activities of the ethanolic extract of *P. carpunya* have also been studied.

* *Corresponding Author*
María Eugenia Rondón, Faculty of Natural Sciences, University of Guayaquil, Guayaquil, Ecuador. Email: rondonr@ula.ve

These activities have been associated with the presence of flavonoids isolated from leaves of this species such as vitexin, isovitexin, rhamnopyranosylvitexin, isoembigenin also the phytosterols and phytol triterpenes (De las Heras *et al*., 1998; Quílez *et al*., 2010). In our continuing interest for the evaluation of biological proprieties and chemical composition of the medicinal plant that grows spontaneously in the Coast of Ecuador, the chemical composition and antibacterial activity of the essential oil of *Piper lenticellosum* C.D.C has been studied.

MATERIALS AND METHODS

Plant Materials

The leaves and spikes of *Piper lenticellosum* C. DC. were collected in July 2015 at Matilde Esther locality, Guayas Province, Ecuador. Botanical identification was carried out by Ricardo Callejas (HUA), and a voucher specimen (code MER01) has been deposited at the GUAY Herbarium, Faculty of Natural Science, University of Guayaquil, Ecuador.

Essential oils Isolation

Fresh leaves and spikes (300 g) were subjected to hydrodistillation for 4 h, using a Clevenger-type apparatus. The oils (6.2 mL, 2.06 %) were dried over anhydrous sodium sulfate and stored in sealed vials at 4°C in the dark until analyzed and tested.

Gas chromatography (GC/FID)

The analyses of the chemical composition of the essential oil were performed on Agilent gas chromatograph (model 6890 N series) equipped with a flame ionization detector (FID) using a non-polar DB-5MS (5% phenyl-methylpolyxilosane) 30 m x 0.25 mm, thickness 0.25 μm capillary column (Agilent 122-5532). An automatic injector (series 7683) in split mode was used. The sample, 1 μL of solution (1/100, v/v, essential oil/dicloromethane), was injected with a split ratio of 1:50. The initial oven temperature was held at 50 °C for 3 minutes, then it was heated to 210 °C with a ramp of 2.5 °C/min, and the temperature was maintained for 3 min until the end. The injector and detector temperatures were 210 °C and 250 °C, respectively. Helium was used as a carried gas at 0.9 mL/min in constant flow mode. The retention index was determined based on the retention times of the standard hydrocarbons TPH-6RPM of CHEM SERVICE C9-C24, which were injected after the oils under the same conditions.

Gas chromatography-mass spectrometry (GC-MS)

The GC-MS analyses were performed using an Agilent gas chromatograph coupled to a mass spectrometer detector (model Agilent series 5973 inlet). The spectrometer was operated at 70 eV, electron multiplier 1600 eV, scan rate: 2 scan/second and mass range: 40–350 m/z. This was provided with a data system MSD-Chemstation D.01.00 SP1. The GC equipped with a DB-5MS 5% phenyl-methylpolysiloxane capillary column (30 m x 0.25 mm x 0.25 μm). The ion source temperature was 250°C. The constituents of the essential oil were identified by comparison of their mass spectra with reference spectra in the computer library (Wiley) and also by comparing their retention indices, with those authentic compounds or data in the literature (Adams, 2007; Joulain and Konig, 1998). The quantitative data were obtained electronically from FID area percentage without the use of correction factor.

Antimicrobial method

The antimicrobial activity was carried out according to the disc diffusion assay described by Velasco *et al*., 2007. The strains were maintained in agar at room temperature. Each bacterial inoculum (2.5 mL) was incubated in Müeller-Hinton broth at 37ºC for 18 hours. The bacterial inoculum was diluted in sterile 0.85% saline to obtain turbidity visually comparable to a McFarland Nº 0.5 standard (10^{6-8} CFU/mL). The minimal inhibitory concentration (MIC) was determined only with microorganisms that displayed inhibitory zones. MIC was determined by dilution of the essential oil in dimethyl sulphoxide (DMSO) pipetting 10 μL of each dilution onto a filter paper disc. Dilutions of the oil within a concentration range of 20- 980 μL/mL were also carried out. MIC was defined as the lowest concentration that inhibited visible bacterial growth (CLSI, 2016). A negative control was also included in the test using a filter paper disc saturated with DMSO (10μL) to check possible activity of this solvent against the assayed bacteria. The experiments were repeated at least twice.

RESULTS AND DISCUSSION

The essential oil was analyzed by Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS). All components (28, representing 98.78 % of the total oil) were characterized by comparison of each MS with the Wiley GC/MS library data also from its retention index (RI). A list of the identified components, along with their percentage of the total oil, is given in Table 1.

The chemical composition of the essential oil was characterized by a high percentage of oxygenated monoterpenes (53.90%) and monoterpenes hydrocarbons (31.11 %) while that the fraction corresponding to sesquiterpenes was very poor (0.46 %). The most abundant compounds in the oil were piperitone (33.97 %), 1,8 cineole (11.92 %), limonene (11.07 %), safrole (8.18 %) and α-pinene (4.49 %). In addition, 8-acetoxy-carvotanacetone (3.83 %), *p*-cymene (3.57 %), α-terpinene (3.11%), β-pinene (2.86%) and ascaridole (2.60%) were also detected. These data collected were relatively discordant with the only report available in the literature on the essential oil of *P. carpunya* (syn. *P. lenticellosum*) from Peruvian Amazon which showed as major compounds α-terpinene (12.1%), *p*-cymene (10.9%), 1,8-cineole (13.0%) and safrole (14.9%) from the leaves, while that α-terpinene (9.8%), p-cymene (7.7%), 1,8-cineole (30.2%) and safrole (32.0%) from the spikes. Piperitone was not observed in the Peruvian species (Vargas *et al*., 2004). These

results evidenced a possible phytochemical intraspecific variability.

Table 1. Chemical composition of the essential oil of *Piper lenticellosum* C.D.C

Peak No	Compounds[a]	%A	RT	KI_{cal}	KI_{tab}
1	α-Thujene	0.22	5.24	924	924
2	α- Pinene	4.49	5.42	930	932
3	Camphene	0.53	5.76	943	946
4	Sabinene	1.80	6.32	962	969
5	β-Pinene	2.86	6.42	965	974
6	β-Myrcene	1.43	6.70	974	988
7	δ-2-Carene	0.22	6.99	993	1001
8	α-Phellandrene	0.87	7.09	996	1002
9	α- Terpinene	3.11	7.42	1005	1014
10	*p*-cymene	3.57	7.64	1013	1020
11	limonene	11.07	7.77	1019	1024
12	1,8-Cineole	11.92	7.87	1023	1026
13	γ -Terpinene	0.94	8.60	1045	1054
14	cis-Sabinenehydrate	0.31	8.87	1056	1065
15	Linalool- L	2.39	9.81	1093	1095
16	Camphor	0.72	11.28	1144	1141
17	Citronellal	0.23	11.48	1150	1148
18	Terpinen-4-ol	0.60	12.31	1175	1174
19	Ascaridole	2.60	14.31	1240	1237
20	Carvone	0.38	14.47	1245	1239
21	Piperitone	33.97	14.93	1260	1249
22	α-Terpinen-7-al	0.78	15.78	1287	1283
23	Safrole	8.18	15.93	1291	1285
24	Carvacrol	0.58	16.28	1302	1298
25	α-Terpinelyl acetate	0.41	17.84	1352	1346
26	*p*-cymen-7-ol-acetate	0.31	20.11	1422	1421
27	Germacrene-D	0.46	21.96	1486	1484
28	8-acetoxy-carvotanacetone	3.83	24.48	1565	1564
	Monoterpene hydrocarbons		31.11		
	Oxygenated monoterpenos		53.90		
	Sesquiterpene hydrocarbons		0.46		
	Oxygenated Sesquiterpenes		0		
	Phenylpropanoids		8.76		
	Other compounds		4.55		
	Total identified		98.78		

[a] Compounds are listed in sequence from DB-5MS column elution
[b] Kovats retention indices (RI) were calculated against C_9 to C_{24} *n*-alkanes Series on a DB-5MS column.

Some chemical polymorphism to *Piper* genus is known. In Bolivia, for example, the main constituent is 1,8-cineol (40%), while that a chemotype frequently found in the Americas the dillapiol is predomint (30-90%) (Guerrini *et al*., 2009). Safrole and other alkylbenzenes are present in percentage significantly high in some species of *Piper* genus such as *P. obliquum* Ruiz & Pav. (safrole, 45.8 %) and *P. aduncum* L. (dillapiol, 45.9%), both species from eastern of Ecuador (Guerrini *et al.*, 2009); *P. hispidinervum* C. DC. (safrole, 85.08 %) (Aumeeruddy-Elalfi *et al*., 2015); *P. betle* L. (safrole, 48.06%) (Telci *et al*., 2010) among others. However, safrole in the essential oil of *P. lenticellosum* was relatively low (8.18%) and dillapiol was not observed.

Additionally, this clear difference between the two essentials oils might be due to climatic conditions. It is well documented that essential oils yield vary considerably depending on the time of the year of plant collection and is also influenced by environmental conditions at the time of harvesting (Juliani *et al*., 2002). However, this species are widely used in traditional medicine in Ecuador, Colombia and Perú (Grandtner and Chevrette, 2013).

Antibacterial activity of *Piper lenticellosum* essential oil was screened against international reference bacterial strains and results obtained are summarized in Table 2. The essential oil showed a significantly inhibition of the growth of the bacterial strains producing a zone of inhibition ranging from 7 to 17 mm with MIC values ranging from 100 to 900 µL/mL. These results showed that the essential oil was most active against *S. aureus*, *E. coli* and *K. pneumoniae* with MIC values ranging 100, 200 and 300 µL/mL, respectively. The antibacterial activity of the essential oil of *P. lenticellosum* could be due to the presence of piperitone, the major component of the oil (33.97%). The antibacterial properties of the piperitone have been reported (Mahboubi and Haghi, 2008) thus as the capacity as nitrofurantoin resistance modulating agent (Shahverdi *et al*., 2015). Additionally, other *Piper* species have showed antibacterial activity against different Gram positive and Gram negative bacteria related at presence to monoterpens as α-pinene and β-pinene (Morales *et al*., 2013); alkylbenzenes as safrole (Guerrini *et al*., 2009) that are also present in the essential oil of *P. lenticellosum*. To the best of our knowledge, this is the first report on the antibacterial activity of the essential oils *P. lenticellosum* from Ecuador. With these results, we hope to contribute to the study of species of the genus *Piper* from Ecuador, of which many are used since ancient times by the Ecuadorian population.

Table 2: Antibacterial activity of the essential oil of *Piper lenticellosum* C.D.C.

Microorganisms	Inhibition Zone (mm)*					MIC (µL/mL)
	Essential oil	Reference compounds				
		OX	VA	CFX	AZT	IMP	
Staphylococcus aureus ATCC (25923)	17	26					100
Enterococcus faecalis ATCC (29212)	7		19				900
Escherichia coli ATCC (25922)	13			20			200
Klebsiella pneumoniae ATCC (23357)	9,5				26		300
Pseudomonas aeruginosa ATCC (27853)	8					24	800

*Inhibition Zone, diameter measured in mm, disc diameter 6 mm, average of two consecutive assays.
OX: Oxacilin® BBL™ (1 µg); VA: Vancomycin® Himedia (30 µg); CFX: Cefuroxime® Oxoid (30 µg); AZT: Aztreonam® Oxoid (30 µg); IMP: Imipenem® BBL™ (10 µg); MIC: Minimal inhibition concentration, concentration range 20 – 980 µL/mL.

CONCLUSION

In the present research, several differences were observed in the composition of *P. lenticellosum* essential oil comparing to *Piper carpunya* (Vargas *et al*., 2004), this might be attributed to geographical environment, seasonality, physiological age of the plant, harvesting time, among other conditions. The essential oil showed a broad spectrum against both Gram positive and Gram negative bacteria attributed to piperitone, mainly. These results may explain the use of these species in the traditional medicine as anti-diarrheal, bronchitis and skin conditions.

It must also consider that these apparent differences in the chemical composition may be due to *P. lenticellosum* should be treated as a related but separate species and not as a synonym for *P. carpunya*. Phylogenetic and molecular studies are recommended for both species.

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