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ARTICULO DE INVESTIGACION

Chemical composition and antibacterial activity of the leaf essential oil of *Eucalyptus globulus* Labill. from two highs of the canton Cañar, Ecuador

Composición química y actividad antibacteriana del aceite esencial de la hoja de *Eucalyptus globulus* Labill. en dos alturas del cantón Cañar, Ecuador

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ABSTRACT

The eucalyptus essential oil has been used in the natural medicine and it has been investigated the medicinal properties of these species that include a wide range of biological activities. The composition of an essential oil can be affected by several factors, such as genetic, ontogenic, morphogenetic and environmental factors. The present work investigated the chemical composition and antibacterial activity of the leaf essential oil of *Eucalyptus globulus* Labill. plants, which grow in Moyancón and Chorocópte regions (1347 and 3191 m height above sea level, respectively) in the canton Cañar, Ecuador. The essential oils were isolated by means of hydrodistillation during two hours and analyzed by gas chromatography coupled to flame ionization and mass selective detectors. The essential oil yields of leaves from Moyancón and Chorocópte regions were 0.17 % and 0.15 % (v/m), respectively. A total of 99 volatile compounds were identified and quantified, where 1,8-cineol and α-pinene were the major compounds. No significant differences in the composition were found by effect of the growth region. The essential oil from Moyancón region showed moderate antibacterial activity against *Staphylococus aureus*, *Streptococus pyogenes* and *Eschericha coli*.

Keywords: eucalyptus, essential oil, chemical composition, antibacterial activity.

RESUMEN

El aceite esencial de eucalipto se ha usado en la medicina natural y se ha investigado las propiedades medicinales de estas especies, que incluyen una amplia gama de actividades biológicas. La composición de un aceite esencial puede estar afectada por varios factores, tales como variación fisiológica, condiciones ambientales, variaciones geográficas, así como factores genéticos y evolutivos. En el presente trabajo se investigó la composición química y actividad antibacteriana del aceite esencial de hojas de *Eucalyptus globulus* Labill. que crece en las regiones de Moyancón y Chorocópte (1347 y 3191 metros sobre el nivel del mar, respectivamente) en el cantón Cañar, Ecuador. Los aceites esenciales fueron aislados mediante hidrodestilación durante dos horas y analizados por cromatografía de gases con detectores de llama de hidrógeno y selectivo de masas. Los rendimientos de aceite esencial para las regiones de Moyancón y Chorocópte fueron 0.17 % y 0.15 % (v/m), respectivamente. Se identificaron y cuantificaron 99 compuestos volátiles, donde predominaron el 1,8-cineol y α-pineno. No se encontraron diferencias significativas en la composición por efecto de la región de crecimiento. El aceite esencial de la región de Moyancón mostró actividad antibacteriana moderada contra *Staphylococus aureus*, *Streptococus pyogenes* y *Eschericha coli*.

Palabras claves: eucalipto, aceite esencial, composición química, actividad antibacticidad.

INTRODUCTION

Infectious diseases represent a continuous and increasing threat to human health and welfare. They are the major causes for vast morbidity and mortality in the world, although developing countries are carrying the major part of the problem (Tariq *et al.*, 2019). The number of infections caused by new, reemerging, or antibiotic resistant pathogens is growing daily, and the increased proportion of patients with immunodeficiency has resulted in an increase of severe and invasive infections (WHO, 2016). Hence, there is an urgent need to find alternative antimicrobial agents for the treatment of resistant pathogenic microorganisms.

Among the alternatives studied, many essential oils have been used in folk medicine throughout the world, and their medicinal properties have been investigated (Saeidnejad & Rajaei, 2015; Ambrosio *et al.*, 2017; Tariq *et al.*, 2019). *Eucalyptus globulus* Labill. (Myrtaceae) is the principal source of eucalyptus leaf oil in the world and exhibit antibacterial, antifungal, analgesic and anti-inflammatory properties and have also been widely used in pharmaceutical, food and cosmetics products (Giles *et al.*, 2010; Damjanović-Vratnica *et al.*, 2011; Mulyaningsih *et al.*, 2011; Elaissi *et al.*, 2011; Tyagi & Malik, 2011; Harkat-Madouri *et al.*, 2015; Luís *et al.*, 2016). The main component of the essential oil is the monoterpene oxide 1,8-cineole, also known as eucalyptol, being the amount of this compound between 44 and 84% and it is known to possess significant antimicrobial activity (Ishnava *et al.*, 2013; Goldbeck *et al.*, 2014).

Generally, it is recognized that plant secondary metabolites do not remain stabilized like other qualitative and quantitative traits. The influencing factors responsible for those fluctuation can be divided into genetic, ontogenic, morphogenetic and environmental factors. All these four principal factors are important in the production or accumulation of plant secondary metabolites and are the reasons of the fluctuations in chemical composition and biological activities (Verma & Shukla, 2015).

Several species of bacteria are pathogenic and cause infectious diseases, such as the Gram-positive bacterium *Staphylococcus aureus*, which is mainly responsible for post-operative wound infection, toxic shock syndrome and food poisoning. *Escherichia coli*, a Gram-negative bacteria, is present in human intestines and causes urinary tract infection, cholecystitis or septicemia. The Gram-positive bacterium *Streptococcus pyogenes* cause a variety of diseases such as streptococcal pharyngitis, rheumatic fever, rheumatic heart disease, and scarlet fever (Reddy *et al.*, 2012).

The present study was undertaken (*i*) to analyze the chemical composition of the leaf essential oil of *Eucalyptus glogulus* Labill. grown in two regions of heights of the canton Cañar and (*ii*) to assess their antimicrobial properties on three clinically significant bacterial strains essential oils.

MATERIALS AND METHODS

Materials

Leaves of eucalyptus were collected in two regions of the canton Cañar in Ecuador: Moyancón (1347 m height above sea level) and parish Chorocópte (3191 m height above sea level), in March 2018. The identity of the plant specimens was confirmed

and registered in the herbarium of the National Park of the Catholic University of Cuenca, where a voucher specimen was deposited (EUC-104).

Essential oil isolation

The fresh leaves (100 g) were submitted to hydrodistillation in a Clevenger-type apparatus for two hours. Isolations were made by duplicate. The essential oils were dried over anhydrous sodium sulfate and stored in hermetically sealed dark-glass containers at -4 °C until further analyses.

Gas chromatography-flame ionization detector and gas-chromatography-mass spectrometry

Analyses of the essential oils were performed by gas chromatography with a flame ionization detector (GC-FID) on an HP-6890 instrument gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a 30 m × 0.25 mm i.d. × 0.25 mm DB-5ms (J & W Scientific, Folsom, CA, USA). The analyses were conducted under the following conditions for both columns: oven temperature program, 70 °C (2 min), 70-240 °C (4 °C/min) and 240 °C (5 min); carrier gas helium flow rate 1 mL/min; injector and detector temperatures 250 °C, injection volume 0.2 µL and split ratio 20:1.

Essential oils were also analyzed by gas chromatography-mass spectrometry (GC-MS) using a GC-MS QP-2010 Ultra (Shimadzu, Kyoto, Japan) fitted with a 30 m × 0.25 mm i.d. × 0.25 mm BP-5 (SGE Analytical Science Pty. Ltd., Victoria, Australia) column. GC parameters were like GC-FID and interface temperature: 250 °C; MS source temperature: 230 °C; MS quadrupole temperature: 150 °C; ionization energy: 70 eV; mass range: 35-350 m/z. The different components were identified using the retention indices and mass spectra. Retention indices, calculated using linear interpolation relative to retention times of C_6-C_{24} of *n*-alkanes, were compared with those standards and data from the literature (Adams, 2001). Mass spectra were compared with corresponding reference standard data reported in the literature (Adams, 2001) and mass spectra from NIST 05, Wiley 6, NBS 75 k, and in-house Flavorlib libraries. In many cases, the essential oils were subject to co-chromatography with authentic compounds. The quantification of compounds was performed using relative percentage abundance and normalization method with correction response factors based on grouping the essential oil components by their functional groups (Costa *et al.*, 2008). Percentage data are the mean values of two injections per sample.

Antibacterial assay

The minimal inhibitory concentration (MIC) of the essential oil was determined by broth microdilution methods (Mulyaningsih *et al.*, 2010). Briefly, the samples were pipetted into 96-well microtiter plates in Mueller Hinton broth (Fluka, Switzerland) followed by a twofold serial dilution. An inoculum suspension was added to give a final concentration of 5×10^5 cfu/mL. After incubation at 37 °C for 24 h, MIC was determined as the lowest concentration without bacterial growth. The minimal bactericidal concentration (MBC) was determined by subculturing 3 µL from each

well without apparent microbial growth on Columbia 5% sheep blood agar and incubated at 36 °C for 24 h. The lowest concentration without apparent microbial growth was taken as the MBC. The experiments were performed in duplicate and repeated twice. Three bacterial strains were used for the antimicrobial studies: two Gram-positive reference strains: *Staphylococus aureus* ATCC 25923 and *Streptococus pyogenes* ATCC 28422 and one clinical isolate *Eschericha coli* (Gram-negative).

RESULTS AND DISCUSSION

The essential oil yields of leaves from Moyancón and Chorocópte regions were 0.17 % and 0.15 % (v/m), respectively. Both essential oils had a spicy aromatic odor. These results were rather small compared to the results reported in the literature where the yield was 1–3% (Giles *et al.*, 2010; Damjanović-Vratnica *et al.*, 2011; Mulyaningsih *et al.*, 2011; Elaissi *et al.*, 2011; Tyagi & Malik, 2011; Ishnava *et al.*, 2013; Goldbeck *et al.*, 2014; Harkat-Madouri *et al.*, 2015). No significant differences were found in the essential oil yields from the two regions of heights of the canton Cañar.

The chemical composition of the leaf oils of *E. globulus* grown in two regions of heights of the canton Cañar was determined by GLC-MS (Table 1). A total of 99 compounds were unambiguously identified, representing 100% of the total composition. Monoterpenoids were predominantly found in both essential oils.

Table 1. Chemical composition of the essential oils from *E. globulus* leaves collected in two heights of the canton Cañar

Compound	LRI	Moyancón	Chorocópte
3-Methylbutan-1-ol	742	tr	tr
2-Methylpropanoic acid	785	tr	tr
3-Methylbutanoic acid	837	tr	tr
(*E*)-3-Hexenol	856	tr	tr
Ethyl 3-methylbutanoate	859	tr	tr
Hexan-1-ol	870	tr	tr
3-Methylbutyl acetate	881	tr	tr
Santolina triene	909	tr	tr
α-Thujene	930	0.1	0.1
α-Pinene	939	12.8	13.1
α-Fenchene	950	tr	tr
Camphene	954	tr	tr
Thuja-2,4(10)-diene	960	tr	0.1
Verbenene	966	tr	tr
Sabinene	973	tr	tr
β-Pinene	978	0.6	0.8
Octan-3-ol	991	tr	tr
Myrcene	997	0.4	0.5
(*Z*)-3-Hexenyl acetate	1005	0.1	0.1
δ-3-Carene	1011	tr	tr
p-Cymene	1023	0.7	0.9
Limonene	1028	1.0	1.4

Table 1. (continued)

Compound	LRI	Moyancón	Chorocópte
1,8-Cineole	1033	67.4	67.7
cis-Linalool oxide (furanoid)	1073	tr	tr
trans-Linalool oxide (furanoid)	1085	tr	tr
p-Cymenene	1093	tr	tr
Linalool	1097	0.1	0.1
3-Methylbutyl 3-methylbutanoate	1104	0.1	0.1
2-Phenylethanol	1108	tr	tr
endo-Fenchol	1115	tr	tr
trans-p-Mentha-2,8-dien-1-ol	1123	0.1	0.1
α-Campholenal	1128	tr	tr
cis-p-mentha-2,8-dien-1-ol	1133	tr	tr
trans-Pinocarveol	1138	0.2	0.2
cis-Verbenol	1141	0.3	0.4
trans-Menthone	1153	0.1	0.1
Isomenthone	1160	tr	tr
Pinocarvone	1165	0.1	0.1
Borneol	1169	0.2	0.2
Terpinen-4-ol	1174	0.3	0.4
Limonen-4-ol	1177	tr	tr
Octanoic acid	1179	tr	tr
3-Oxocineol	1181	tr	0.1
p-Methylacetophenone	1183	tr	tr
p-Cymen-8-ol	1185	0.1	0.1
Cryptone	1187	0.1	tr
trans-p-Mentha-1(7),8-dien-2-ol	1189	tr	tr
α-Terpineol	1191	1.7	1.8
1-Phenylethyl acetate	1194	tr	tr
cis-Piperitol	1196	tr	tr
Myrtenal	1199	0.2	0.2
Verbenone	1205	0.3	0.3
trans-Carveol	1218	0.1	0.1
1,8-Epoxy-*p*-menthan-2-ol	1225	tr	tr
cis-Carveol	1229	tr	tr
Nerol	1231	tr	tr
(*Z*)-3-Hexenyl 2-methylbutanoate	1234	tr	tr
Pulegone	1237	0.2	0.2
Carvone	1244	0.2	0.2
Geraniol	1253	0.4	0.4
2-Phenylethyl acetate	1258	tr	tr
Geranial	1267	0.1	0.1
Perilla aldehyde	1272	0.1	0.1
Isobornyl acetate	1284	tr	tr
p-Cymen-7-ol	1291	tr	tr
3-Methoxy-acetophenone	1298	tr	tr
8-*p*-Menthene-1,2-diol	1324	tr	0.1
exo-2-Hydroxycineole acetate	1344	0.1	0.1
α-Terpinyl acetate	1349	1.8	1.6
Geranic acid	1355	tr	tr

Table 1. (continued)

Compound	LRI	Moyancón	Chorocópte
Eugenol	1359	tr	tr
Neryl acetate	1363	tr	tr
Decanoic acid	1373	tr	tr
Isoledene	1376	0.1	0.1
α-Copaene	1379	0.1	0.1
Geranyl acetate	1383	0.5	0.4
β-Bourbonene	1388	tr	tr
Benzyl 3-methylbutanoate	1395	tr	tr
α-Gurjunene	1410	0.4	0.3
8-Hydroxycarvotanacetone	1414	tr	0.1
(*E*)-Caryophyllene	1421	3.3	3.0
α-Humulene	1454	tr	tr
allo-Aromadendrene	1460	0.7	0.5
ar-Curcumene	1482	tr	tr
2-Phenylethyl 3-methylbutanoate	1492	0.2	0.1
Viridiflorene	1497	0.2	0.2
α-Muurolene	1502	tr	tr
γ-Cadinene	1514	tr	tr
cis-Calamenene	1540	0.1	tr
α-Calacorene	1547	tr	tr
epi-Globulol	1563	0.4	0.3
Carvotacetone acetate	1567	0.1	tr
Globulol	1586	2.0	1.8
Viridiflorol	1595	0.6	0.5
Rosifoliol	1602	0.2	0.1
Humulene epoxide II	1608	tr	tr
β-Eudesmol	1651	0.1	0.1
α-Eudesmol	1654	0.1	0.1
δ-Dodecalactone	1707	0.1	0.1

LRI: Lineal retention index in DB-5ms column. tr: <0.1%.

The essential oils contained mainly 1,8-cineole (67.4 and 67.6% in Moyancón and Chorocópte regions, respectively) and α-pinene (12.8 and 13.1%) were the main compounds. The hydrocarbon (*E*)-caryophyllene was the major sesquiterpene. The leaf oil of *E. globulus* is well known to be a 1,8-cineole-rich oil (Giles *et al.*, 2010; Damjanović-Vratnica *et al.*, 2011; Mulyaningsih *et al.*, 2011; Elaissi *et al.*, 2011; Tyagi & Malik, 2011; Ishnava *et al.*, 2013; Goldbeck *et al.*, 2014; Harkat-Madouri *et al.*, 2015; Luís *et al.*, 2016). Some differences can occur in composition of essential oils from the same species probably due to genetic variation and different environmental factors, such as climate, harvesting seasons and geographical location (Verma & Shukla, 2015). No significant differences were found in the chemical composition of the essential oils from the two regions of heights of the canton Cañar.

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the essential oils from *E. globulus* leaves collected in the canton Cañar

Strain	MIC (mg/mL)	MBC (mg/mL)
Staphylococus aureus	2.87	3.10
Streptococus pyogenes	4.89	5.87
Eschericha coli	1.90	2.21

Considering that both essential oils have the same composition, only the oil sample from Moyancón area was evaluated for its antibacterial activity. The MIC and MBC values of the eucalypt essential oil are presented in Table 2 and vary from 1.90 to 5.87 mg/mL. The essential oil showed moderate activities against the evaluated strains because, in general, the MIC and MBC were > 2 mg/mL. Mulyaningsih *et al.* (2011) reported a MIC value of > 4 mg/mL for *E. coli*, while Damjanović-Vratnica *et al.* (2011) found lower MIC and MBC values for *S. aureus* (0.09 and 0.18 mg/mL, respectively), *S. pyogenes* (0.09 and 0.09 mg/mL) and *E. coli* (0.09 and 0.18 mg/mL). On the other hand, Tyagi and Malik (2011) reported MIC and MBC values of 4.5 and 9 mg/mL for *E. coli*.

CONCLUSIONS

The leaf essential oils of *Eucalyptus globulus* Labill. plants, which grow to 1347 and 3191 m height above sea level in the canton Cañar, Ecuador, had the same chemical composition, which was dominated mainly by 1,8-cineole (>67% of total composition). No significant differences were found between both chemical composition of the essential oils. The essential oil showed moderate antibacterial activity against *Staphylococus aureus*, *Streptococus pyogenes* and *Eschericha coli*.

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