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Phytochemical analysis and biological potential of Argentinian plant essential oils and extracts

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ABSTRACT

Our aim is to characterize the chemical composition, antimicrobial, antioxidant and anti-inflammatory potentials of the essential oils (EOs) and ethanolic extracts (EEs) of five northwest native plants in Argentina. The EOs and EEs were obtained from *Lippia turbinata*, *Clinopodium gilliesii, Lippia integrifolia, Zuccagnia punctata*, and *Senecio subulatus* var. *salsus*. EOs and EEs phytochemical composition were determined by GC-MS analysis and spectrophotometric methods. Antibacterial activity was assessed against Gram-negative and -positive pathogenic bacteria. Antioxidant activity was evaluated by DPPH radical scavenging assay and anti-inflammatory potential was determined by cyclooxygenase (COX-2) inhibition assay. EOs and EEs of all assayed plant species showed weak antibacterial effect. The EEs had stronger scavenging activity than the EOs. The best results were achieved for *Z. punctata* EE followed by *L. turbinata* and *C. gilliesii* EEs. The EOs exhibited greater inhibitory activity towards the COX-2 than EEs. *C. gilliesii* and *L. integrifolia* EOs showed the highest COX-2 inhibitory activity. These results would indicate that antioxidant activity is concentrated in the non-volatile fraction of the plants whether the anti-inflammatory activity is in the volatile one. This work contributes to knowledge of biological properties of plants from our region and could help to discover compounds with potential therapeutic uses.

Keywords: Antioxidant activity, Anti-inflammatory activity, Chemical composition, Argentine northwestern plants

INTRODUCTION

Medicinal and aromatic plants have been used extensively for therapeutic and flavoring purposes across history and cultures, and still represent a valuable source of new biologically active compounds for pharmaceutical and food industries (Alonso and Desmarchelier 2015).

The use of natural products as antimicrobial, antioxidant and anti-inflammatory agents, especially those derived from plants, has been of particular interest since natural products have historically made a major contribution to pharmacotherapy (Atanasov et al. 2021).

Many factors can affect the recovery of bioactive compounds from plant materials. The use of different extraction techniques modulates the plant samples chemical composition and, consequently, their biological properties. Most of these techniques are based on the extracting power of different solvents and the application of heat and/or mixing (Cabana et al. 2013; Azmir et al. 2013). Hydrodistillation and maceration are two of the conventional extraction techniques. Here, we used both techniques to obtain essential oils (EOs) and ethanolic extracts (EEs) from five native plants extensively used in Argentine northwestern as aromatic herbs as well as for medicinal purposes.

The plants used were: *Lippia turbinata* Griseb (Verbenaceae), *Clinopodium gilliesii* (Benth.) Kuntze (Lamiaceae), *Lippia integrifolia* (Griseb.) Hieron (Verbenaceae), *Zuccagnia punctata* Cav. (Fabaceae), and *Senecio subulatus* D. Don ex Hook. & Arn. variety *salsus* (Griseb.) Cabrera (Asteraceae).

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Lippia turbinata, *C. gilliesii*, and *L. integrifolia* are aromatic shrubs commonly known as "poleo", "muña-muña" and "incayuyo", respectively. Infusions of their aerial parts are traditionally used as a digestive. Besides, *L. turbinata* and *L. integrifolia* have been included in the Argentine Food Code as herbs for infusions and are used industrially in the production of non-alcoholic beverages, appetizers and teas (Alonso and Desmarchelier 2015). Several previous studies have described the chemical composition of the EOs of these three species while a few ones have investigated their biological properties. The antibacterial (Luna et al. 2008; Lima et al. 2011; Pérez Zamora et al. 2016) and antioxidant (Quiroga et al. 2013; Cabana et al. 2013; Barbieri et al. 2016) activities were reported for the three EOs while anti-inflammatory activity has been informed only for the *C. gilliesii* EO (Reynoso et al. 2018).

Zuccagnia punctata known as ''jarilla macho'' or ''pus-pus'' is endemic of central and western semi-arid regions of Argentina. It is currently used in traditional medicine as an antiseptic against bacterial and fungal infections (Álvarez et al. 2012; Zampini et al. 2012). The EE as well as EO of this plant has been reported to possess antimicrobial activity (Zampini et al. 2005, 2012; Álvarez et al. 2012). The antioxidant (Morán Vieyra et al. 2009) and anti-inflammatory (Nuño et al. 2018) capacity of flavonoids isolated from *Z. punctacta* have also been demonstrated.

Senecio subulatus commonly known as "romerillo" grows in Puneña and pre-puneña areas from Argentina. It is well known to be toxic to livestock probably due to their pyrrolizidine alkaloids content (Pestchanker et al. 1985; Pestchanker and Giordano 1986). Although there are no reports on the biological activity of this species, there is information regarding other *Senecio* species from Argentina such as *S. mustersii*, *S. subpanduratus* (Arancibia et al. 2010) and *S. graveolens* (Pérez et al. 1999) with antimicrobial activity.

Since little information has been found about phytochemical composition and biological activity of the species from La Rioja and Catamarca provinces (Northwest Argentina), our main purpose was to perform a phytochemical characterization and identification of bioactivity in two different extracts (EO and EE) obtained from these five native plants. This is the first report on the chemical composition of EOs from *Z. punctata* and *S. subulatus* var. *salsus* collected in Dpto. Chilecito (La Rioja) and Dpto Belén (Catamarca), Argentina, respectively.

MATERIAL AND METHODS

Plant material

Lippia turbinata, Clinopodium gilliesii, Lippia integrifolia, and *Zuccagnia punctata* plants were collected from Valle Antinaco-Los Colorados, Dpto. Chilecito, province of La Rioja, Argentina. *Senecio subulatus* var. *salsus* was collected from Villa Vil, Dpto. Belén, province of Catamarca, Argentina. The plant material was identified by Lic. Gloria Jaime and Lic. María José Loyola. Voucher specimens have been deposited in Herbarium of Universidad Nacional de Chilecito. The plants were identified being compared with voucher specimens deposited at the Fundación Miguel Lillo Herbarium (LIL,Tucumán, Argentina) and the Museo Botánico de Córdoba Herbarium (CORD, Córdoba, Argentina). The voucher data of Cfr (examined reference material) compared with collected material are the following: *L. turbinata* Griseb. Cfr *L. turbinate* Griseb. LIL 27589, *C. gilliesii* (Benth.) Kuntze Cfr Syn. *Satureja parvifolia* (Phil.) Mold. LIL 420243, *L. integrifolia* (Griseb.) Hieron. Cfr *L. integrifolia* (Griseb.) Hieron. LIL 609723, *Z. punctata Cav.* Cfr *Z. punctata Cav.* LIL 605935, and *S. subulatus* var. salsus (Griseb.) Cabrera Cfr *S. subulatus* var. salsus (Griseb.) Cabrera CORD 6824.

The air-dried aerial parts of the plants were used to obtain the EOs and EEs.

Essential oils isolation

Extractions of EOs were carried out by hydrodistillation in a Clevenger apparatus for 3 h. The EOs obtained were stored at -20 °C for subsequent experiments. Sample yields were the following: 0.87% for *L. turbinata*, 2.78% for *C. gilliesii*, 2.79% for *L. integrifolia,* 0.21% for *Z. punctate*, and 1.07% for *S. subulatus* var. *salsus*.

Ethanolic extract preparation

Plant material was extracted using a 24-hour maceration with ethanol 96° GL. Then, the EEs were filtered and evaporated under reduced pressure using a rotary evaporator. The waxes were removed by precipitation from a 50% (v/v) ethanol-water solution of the extracts. The EEs were dissolved in 50% ethanol and shaken using a 40 kHz ultrasonic cleaning bath for 60 min. Subsequently, they were centrifuged (560 ×*g*, 10 min) and the separated supernatants were evaporated. Finally, the samples were stored in the dark at room temperature.

Chemical characterization of the essential oils: GC-MS analysis

The volatile constituents of the EOs were analyzed using a gas chromatography-mass spectrometry (GC-MS) carried out on a Trace GC Ultra

- Polaris Q GCMS (Thermo Electron Corporation) fitted with a DB 5-fused silica column (30 m x 0.25 mm i.d., a film thickness of 0.25 µm). The initial oven temperature was set at 60 ºC, then it was increased to 250 ºC at 3 ºC/min, and the final temperature was maintained for 5 min. Carrier gas, helium, was adjusted to a linear velocity of 34 cm/s (1 ml/min). The injector temperature was 250 ºC, the detector temperature was 220 °C and an injection volume of 1 µl with a split ratio of 1:10 was used.

The identity of the oil components was established by comparison of their MS spectra with those reported in Nist08 library and the relative percentage of the individual compounds in each EO was calculated using the total ion current from the MS detector signal. Retention indices were calculated in relation to C8-C24 n-alkanes standards and compared with those previously reported in the literature.

Extract phytochemical analysis

Total phenolic content

Total phenolic extract content was evaluated by the spectrophotometric Folin-Ciocalteu method (Singleton et al. 1999) using gallic acid as reference compound. Stock extract solutions were prepared in 10% ethanol at 10 mg/ml and then diluted as many times as needed with distilled water.

Samples (0.5 ml of different dilutions) were mixed with 0.1 ml of 0.2 N Folin-Ciocalteu reagent (Sigma–Aldrich) for 2 min and 0.4 ml of 15.9% sodium carbonate were then added. The absorbance of reaction was recorded at 765 nm after incubation at room temperature for 20 min.

Total phenolic content was calculated from a gallic acid calibration curve and the results were expressed as mg of gallic acid equivalents (GAE)/g of extract.

Total flavonoid content

Flavonoids were estimated using a spectrophotometric assay based on aluminum chloride complex formation. Flavonoid content was measured as described by Ordóñez et al. (2010). Briefly, 100 µl of serial dilutions from the extract stock solution were mixed with 100 µl of a solution of aluminum chloride (Anedra, Argentina) in ethanol (2% w/v). After 1 h at room temperature, the absorbance was measured at 420 nm in a microplate reader (Multiskan Go, Thermo Scientific, USA). Total flavonoid content was calculated from a calibration curve of quercetin and the results were expressed as mg of quercetin equivalents (QE)/g of extract.

Biological potential

The biological activities of the EOs and EEs were determined using antimicrobial, antioxidant, and enzyme inhibition assays.

Antimicrobial assay

EOs and EEs antibacterial activity was assessed against Gram-negative and -positive pathogenic bacteria using the agar dilution method with minor modifications (Clinical and Laboratory Standards Institute (CLSI) 2012). *Escherichia coli* and *Salmonella typhimurium* were from American Type Culture Collection (ATCC 8739 and ATCC 14028, respectively). *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae* were isolated from clinic samples at the Instituto de Microbiología 'Luis Verna' of the Universidad Nacional de Tucumán and the Children's Hospital, Tucuman, Argentina.

The microorganisms were maintained at −70 °C in Brain Heart Infusion (BHI, Britania, Argentina) containing 30% (v/v) glycerol. Bacterial inocula were prepared from an overnight culture in BHI agar (*E. coli, S. typhimurium, P. aeruginosa* and *S. aureus*) or in Columbia agar 5% sheep blood (*S. agalactiae* and *S. pneumoniae*). Microorganism suspensions were adjusted in a spectrophotometer with sterile 0.9% NaCl solution to give a final cell suspension of 10^8 CFU/ml (colony forming units/ml). Then, the suspensions were diluted to obtain a concentration of 10^7 CFU/ml.

Stock solutions of EOs in DMSO-water and EEs in ethanol-water were diluted to give serial two-fold dilutions. The same volume (1 ml) of each sample dilution (EO or EE) was added to 5 ml of Mueller–Hinton agar (MHA) to obtain final concentrations ranging from 5-0.31 mg/ml. The final concentration of DMSO or ethanol 96° in the assays did not exceed 2.5%. After cooling and drying, the plates were inoculated in spots with 3 µl of each bacterial cell suspension (10^4 CFU per spot). Growth control for each tested organism, solvent control (DMSO or ethanol 96°), positive controls (chloramphenicol and streptomycin) and EO and EE sterility controls were included. The plates were incubated for 20 h at 35 °C. The minimum inhibitory concentration (MIC) values were defined as the lowest EO or extract concentration showing no visible bacterial growth after the incubation time.

DPPH radical scavenging assay

Antioxidant activity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich) assay (Ordóñez et al. 2010). Briefly, 0.5 mL of DPPH solution (300 µM in methanol) was added to 1.5 ml of the samples previously dissolved in ethanol (concentrations range: 200–5000 µg/ml for EOs and 10-400 µg/ml for EEs). After shaking, the

mixture was incubated for 20 min in darkness at room temperature and then absorbance was measured at 515 nm. The SC_{50} values (concentration of sample required to scavenge 50% DPPH radicals) were calculated using the regression equation prepared from the different sample concentrations. Butylated hydroxytoluene (BHT, Sigma-Aldrich) and ascorbic acid (Sigma-Aldrich) were used as reference compounds.

Cyclooxygenase (COX) inhibition assay

Inhibitory activity on human COX-2 was measured by using a COX-2 Inhibitor Screening Assay Kit (No. 701080, Cayman Chemical, USA) following the manufacturer instruction. This assay is based on measuring prostaglandins (PGs) by enzyme immunoassay (ELISA). COX catalyzes the conversion of arachidonic acid to PGH2 which is reduced to PGF2α and quantified via competitive ELISA.

The inhibitory assays were performed in the presence of EOs (150 and 300 µg/ml) or EEs (50 and 150 µg/ml). The EO and EE concentrations used in COX-2 Inhibition assay were selected according to the results of DPPH scavenging test. Indomethacin (6 µM=2.15 and 12 µM=4.3 µg/ml) was used as reference compound. Pre-incubation time between enzyme and inhibitors was 10 min at 37 °C. The assay for obtaining 100% COX activity was performed with DMSO as solvent control. Enzyme control was performed with COXs that had been inactivated by placing them in boiling water (background values). The ability of the test compounds to inhibit the COX-2 (anti-inflammatory effect) was determined by calculating percent inhibition of PGF2α production. The percentage of COX inhibition was calculated according to the following equation:

Inhibition (%) = (100% COX – Inhibitor sample)/ 100% COX *100.

100% COX is the activity of enzyme in absence of inhibitors and inhibitor sample is the activity of the enzyme in the presence of the EOs, EEs or indomethacin.

Statistical analysis

Experiments were done in duplicate and repeated at least twice. The data are presented as mean ± standard deviation. Statistical significance was analyzed by the analysis of variance followed by the Tukey test (MINITAB software version 15 for Windows, SPSS Inc., Chicago, IL). *p*-values < 0.05 were regarded as significant. The Pearson's correlation coefficients among phenolic or flavonoid content and radical scavenging activity were performed using InfoStat software (version 2017, InfoStat FCA Group, UNC, Argentina).

RESULTS AND DISCUSSION

Phytochemical composition

GC-MS analysis

We report the composition of the EO of the aforementioned plants from northwest Argentina where the climatic conditions are very hot and dry. The retention indices, percentage, composition, and identification for the EO of *L. turbinata*, *C. gilliesii, L. integrifolia, Z. punctate*, and *S. subulatus* var. *salsus* are given in Table 1. The identification of the constituents of the EOs of the species under study constituted 93.2 - 29.8% of the total oil.

The most abundant constituents in the *L. turbinata* EO were found to be limonene, piperitenone oxide, and spathulenol. Analyzed EO mainly consisted of monoterpenes (47.9%) followed by oxygenated monoterpenes (36.1%), oxygenated sesquiterpenes (6.2%), and sesquiterpenes (3,0%). Eleven compounds, 93.2% of the total, were identified in the oil.

Clinopodium gilliesii EO is mainly characterized by pulegone, piperitenone oxide, isopulegone, and isomenthone while all the other constituents were present in low amount. Oxygenated monoterpenes represented the 88.7% of the analyzed EO, while sesquiterpenes represented 1.2%, and aliphatic compounds the 1.1%.

The oil of *L. integrifolia* is mainly characterized by *trans*-davanone and spathulenol, while all the other metabolites were present in a low relative abundance. Oxygenated sesquiterpenes (33.9%) were the main constituents of this EO, followed by monoterpenes (11.8%), oxygenated monoterpenes (7.1%), and sesquiterpenes (5.8%).

The major constituents of *Z. punctata* EO were 5,6-dehydrocamphor and *p*-cymene. Oxygenated monoterpenes (26.0%) were the main constituents of the EO, followed by monoterpenes (17.1%) and sesquiterpenes (9.9%).

Senecio subulatus var. *salsus* EO was mainly characterized by spathulenol, being oxygenated sesquiterpenes the main constituent of the EO (22.4%). Monoterpenes and oxygenated monoterpenes represented the 6.2 and 1.2%, respectively.

Table 1. Main components of the essential oils from *Lippia turbinata, Clinopodium gilliesii, Lippia integrifolia, Zuccagnia punctate*, and *Senecio subulatus* variety *salsus*.

Compound		RI *	(%)
	L. turbinata		
Monoterpene	Limonene	1023	47.9
Oxygenated monoterpene	α-Thujone	1080	0.4
Oxygenated monoterpene	*cis*-2-Menthenol	1103	0.7
Oxygenated monoterpene	Cumin Aldehyde	1203	0.6
Oxygenated monoterpene	Carvone	1212	0.7
Oxygenated monoterpene	Bornyl Acetate	1260	0.7
Oxygenated monoterpene	Piperitenone	1304	1.4
Oxygenated monoterpene	Piperitenone Oxide	1337	30.7
Oxygenated monoterpene	Methyl Eugenol	1374	0.9
Sesquiterpene	β-Caryophyllene	1401	3.0
Oxygenated Sesquiterpene	Spathulenol	1549	6.2
Total			93.2
	C. gilliesii		
Aliphatic ketone	Octen-2-one<(3E)	1020	1.1
Oxygenated monoterpene	Linalool	1088	0.5
Oxygenated monoterpene	Menthone	1129	5.8
Oxygenated monoterpene	Isomenthone	1137	10.9
Oxygenated monoterpene	Isopulegone	1147	13.0
Oxygenated monoterpene	Pulegone	1211	40.5
Oxygenated monoterpene	Piperitenone	1304	2.9
Oxygenated monoterpene	Piperitenone Oxide	1329	13.5
Sesquiterpene	β-Caryophyllene	1402	1.2
Oxygenated Sesquiterpene	Spathulenol	1551	1.6
Total			91.0
	L. integrifolia		
Monoterpene	α-Thujene	918	0.3
Monoterpene	α-Pinene	925	2.7
Monoterpene	Sabinene	960	1.2
Monoterpene	Myrcene	978	0.3
Monoterpene	α-terpinene	1006	0.2
Monoterpene	o-Cymene	1010	4.3
Monoterpene	Limonene	1017	2.4
Monoterpene	γ-Terpinene	1044	0.4
Oxygenated monoterpene	*cis*-Sabinene hydrate	1052	1.3
Oxygenated monoterpene	*cis-p*-Mentha-2,8-dien-1-ol	1108	0.2
Oxygenated monoterpene	*trans-p*-Mentha-2-en-1-ol	1124	0.3
Oxygenated monoterpene	*cis*-Verbenol	1128	1.2
Oxygenated monoterpene	Borneol	1150	0.5
Oxygenated monoterpene	Terpinen-4-ol	1161	2.1
Oxygenated monoterpene	Verbenone	1177	1.0
Oxygenated monoterpene	Pulegone	1210	0.5
Sesquiterpene	β-Caryophyllene	1401	3.1
Sesquiterpene	*cis*-Calamenene	1498	2.7

Table 1. continued

Compound		RI *	(%)
	L. turbinata		
Oxygenated Sesquiterpene	*trans*-davanone	1542	24.7
Oxygenated Sesquiterpene	Spathulenol	1554	9.2
Total			58.6
	Z. punctata		
Monoterpene	α-Pinene	924	1.9
Monoterpene	Thuja-2,4(10)-diene	939	2.6
Monoterpene	α-Phellandrene	992	1.5
Monoterpene	*p*-Cymene	1007	8.8
Monoterpene	Limonene	1014	2.3
Oxygenated monoterpene	(−)-5,6-dehydrocamphor	1064	20.5
Oxygenated monoterpene	Camphenone, 6-	1067	1.8
Oxygenated monoterpene	Linalool	1082	5.1
Oxygenated monoterpene	Terpinen-4-ol	1154	1.6
Sesquiterpene	β-Caryophyllene	1396	0.4
Sesquiterpene	γ-Muurolene	1474	0.7
Sesquiterpene	δ-Cadinene	1495	4.5
Sesquiterpene	α-Calacorene	1507	0.8
Sesquiterpene	Rosiofoliol	1572	3.5
Total			56.0
	S. subulatus* var. *salsus		
Monoterpene	α-Pinene	923	0.4
Monoterpene	Sabinene	961	0.3
Monoterpene	*o*-Cymene	1007	5.1
Monoterpene	Limonene	1014	0.4
Oxygenated monoterpene	Terpinen-4-ol	1165	0.7
Oxygenated monoterpene	Methyl Eugenol	1367	0.5
Sesquiterpene	Spathulenol	1548	22.4
Total			29.8

* RI: Retention index

Chemical constituents of *L. turbinata* EO are mostly in agreement with those previously reported for a collection from Cordoba Province (Argentina) (Velasco-Negueruela et al. 1993). However, some important differences were detected. While the main constituent in the vegetable material from Cordoba province was α-thujone, our collection, in the present investigation as well as in a previous one from 2016 (Barbieri et al. 2016), presented limonene and piperitenone oxide as the main constituents. In agreement with our results, Juliani et al. (2004) also have reported limonene and piperitenone oxide as main metabolites of *L. turbinata* EO from Los Llanos region (La Rioja Province), which suggested that the collection from Cordoba belonged to a different chemo-type.

Clinopodium gilliessi EO composition is in agreement with our previous investigation (Barbieri et al. 2016), also is most in agreement with another collection from La Rioja Province, Argentina (Reynoso et al. 2018). However, a previous investigation of a San Juan Province collection gave piperitone as main constituent (Luna et al. 2008). In order to unambiguously identify pulegone on *C. gilliessi* EO, an aliquot of this oil was analyzed by NMR. Based on the obtained ^{1}H NMR spectrum was able to unequivocally identify the main constituent as pulegone due to diagnostic signals at 1.74 and 1.94 ppm, corresponding to methyls groups over a double bond (data not shown). Pulegone and piperitone share the same biosynthetic route and both compounds could be synthesized via isopiperitenone (Mahmoud and Croteau 2003). Then, the *C. gilliesi* collection from San Juan could belong

to a different chemo-type where piperitone synthesis was favored.

Based on the presence of *trans*-davanone and spathulenol as the majority compounds, it is possible to locate our collection of *L. integrifolia* into the 'spathulenol/bicyclogermacrene chemo-type' previously described by Marcial et al. (2016) where thirty-one wild populations of *L. integrifolia* covering most of its natural range were analyzed and located in five different chemo-types.

Zuccagnia punctata EO content is in agreement with previous reports for La Rioja Province collections (Álvarez et al. 2012) in which 5,6-dehydrocamphor was also the main compound and oxygenated monoterpenes where the major constituents.

There is a lack of information regarding *S. subulatus* EO. A previous investigation of *S. subulatus* var. *salsus* collected in Jujuy Province (pre puneña phytogeographic area) (Dambolena et al. 2008) gave *p*-cymene and β-pinene as main constituents. Since our collection presented spathulenol as majoritary compound, probably we face a different chemo-type. However, a bigger number of *S. subulatus* samples from different locations are needed to carry on further investigations.

Extract phytochemical analysis

Significant differences were found in phenolic content among the different EEs (Figure 1A). The higher phenolic content was observed in *Z. punctata* EE and followed by *C. gilliesii* EE, *L. turbinata* EE, *L. integrifolia* EE, and *S. subulatus* var. *salsus* EE (Figure 1A).

The flavonoid content in EEs ranged from 3 to 265 mg QE/g. *Z. punctata* EE had the greatest flavonoid content while the lowest flavonoid content was found in *S. subulatus* EE (Figure 1B).

EOs and EEs antibacterial activity

Antimicrobial effects, expressed as MIC of each EO and EE against tested Gram-positive and -negative bacteria is given in Table 2.

Clinopodium gilliesii and *L. integrifolia* EOs were active against the three assayed Gram-positive bacteria. The *L. integrifolia* EO was more effective than the *C. gilliesii* EO against *S. agalactiae* and *S. pneumoniae*, which can be evidenced by their MIC values (Table 2). *C. gilliesii* EO also exhibited antibacterial activity against *E. coli* and *S. typhimurium* with the highest MIC values of 5 mg/ml. The *S. subulatus* var. *salsus* EO only showed inhibitory effect on *S. aureus* (Table 2). In the range of concentrations tested, *L. turbinata* and *Z. punctata* EOs showed no antibacterial activity.

According to Luna et al. (2008), the *C. gilliesii* EO showed inhibition values between 1.2-1.6 mg/ml against *E. coli*, *Salmonella* sp and *S. aureus*. On the other hand, Lima et al. (2011) reported that the *L. integrifolia* EO presented moderate activity against the same pathogenic bacteria with MIC values between 0.5-1 mg/ml. Comparing our results with these two works, the antibacterial activity of *C. gilliesii* and *L. integrifolia* EOs observed herein was weaker than that found by these authors.

Regarding EOs components, the antibacterial properties of the piperitone have been

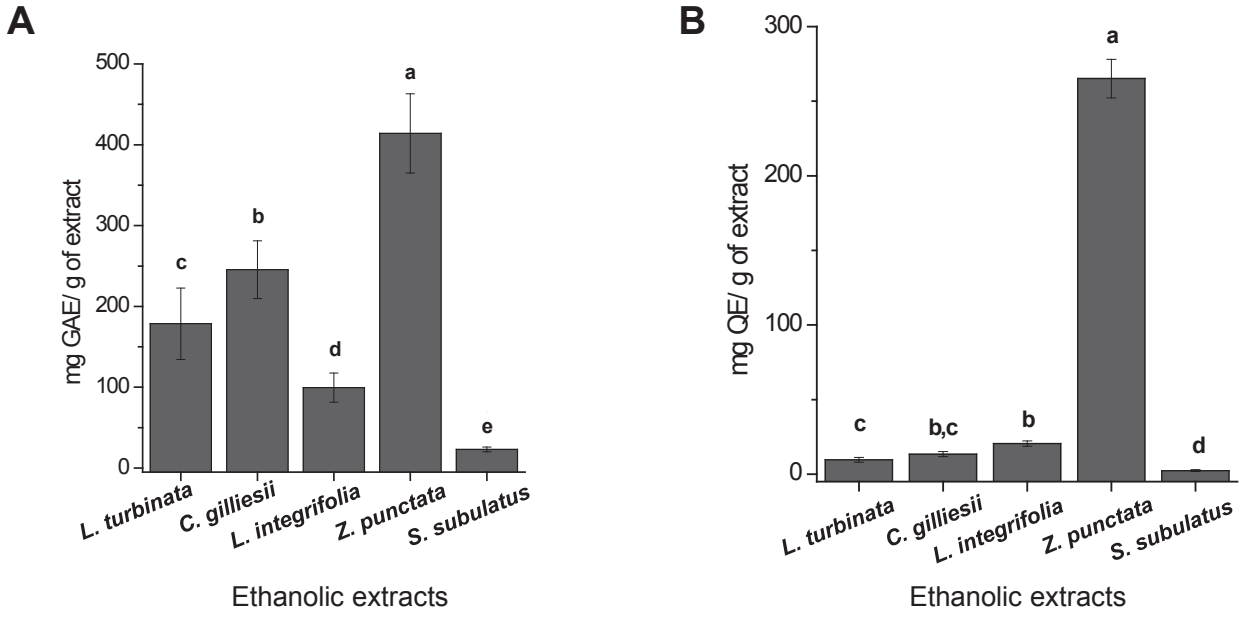

Figure 1. Total phenolic compounds (A) and flavonoid contents (B) of ethanolic extracts. Data are the mean ± SD. Means without a common letter (a, b, c, d) differ (P<0.05)

demonstrated (Shahverdi et al. 2004; Yang et al. 2015). Thus, the differences in the content of this monoterpene between the *C. gilliesii* EO from San Juan (Luna et al. 2008) and ours could explain the differences in their antibacterial activity. In this sense, the differences in MIC values of the EOs may arise from variation in their chemical constituents (chemo-types) as well as the microbiological methods used.

Of the five types of extracts, the best antimicrobial effect on both Gram-positive and -negative bacteria was obtained with the *Z. punctata* EE (Table 2). The MIC values of this extract against Gram-positive bacteria were lower than those against Gram-negative bacteria. *S. aureus* and *S. pneumoniae* were the most sensitive to *Z. punctata* EE (MIC = 1.25 mg/ml) (Table 2). *L. turbinata* EE also showed activity against *S. aureus* while *C. gilliesii*, *L. integrifolia*, and *S. subulatus* var. *salsus* EEs showed no activity against the assayed microorganisms (Table 2).

The anti-staphylococcal and anti-streptococcal activity observed for *Z. punctata* EE is in agreement with the reported by Zampini et al. (2012) and Nuño et al. (2018). They concluded that *Z. punctata* antibacterial activity might be related to the action of 2',4'-dihydroxychalcone and 7-hydroxyflavanone present in the extract. Flavonoids are well documented for their antibacterial properties in both Gram-positive and -negative bacteria (Cushnie and Lamb 2011; Farhadi et al. 2019). In this sense, the antibacterial activity of *Z. punctata* EE might be due to their high concentration of flavonoids. The absence or low activity presented by the other studied EEs could be explained by their low flavonoids content (Figure 1B).

EOs and EEs antioxidant activity

DPPH assay is one of the most extensively used assay to evaluate antioxidant activity. The method is sensitive, requires small sample amounts and allows testing both hydrophilic and lipophilic compounds. EO and EE DPPH results are presented as SC_{50} values in Table 3.

A lower SC_{50} indicates a higher antioxidant activity. The best DPPH radical scavenging activity was shown by *L. turbinata* EO. The other evaluated EOs showed low potency as antioxidants (Table 3).

All the tested EEs showed a stronger antioxidant effect than the EOs with SC_{50} values between 18 and 255 µg/ml. The *Z. punctata* EE presented the best antiradical activity, being this activity higher to that of the BHT reference antioxi-

Table 2. Antibacterial activity of essential oils and ethanolic extracts from *Lippia turbinata, Clinopodium gilliesii, Lippia integrifolia, Zuccagnia punctate*, and *Senecio subulatus* var. *salsus.*

	Gram-positive			Gram-negative		
	S. aureus	***S. agalactiae***	***S. pneumoniae***	***E. coli***	***S. typhimurium***	***P. aeruginosa***
Essential oils MIC (mg/ml)						
L. turbinata	ND	ND	ND	ND	ND	ND
C. gilliesii	5	5	5	5	5	ND
L. integrifolia	5	2.5	2.5	ND	ND	ND
Z. punctata	ND	ND	ND	ND	ND	ND
S. subulatus	2.5	ND	ND	ND	ND	ND
Ethanolic extracts MIC (mg/ml)						
L. turbinata	5	ND	ND	ND	ND	ND
C. gilliesii	ND	ND	ND	ND	ND	ND
L. integrifolia	ND	ND	ND	ND	ND	ND
Z. punctata	1.25	2.5	1.25	5	5	5
S. subulatus	ND	ND	ND	ND	ND	ND
Controls MIC (µg/ml)						
STR	<18.75	75	75	<18.75	<18.75	37.5
CHL	200	200	200	200	200	ND

ND: not determined. In the range of concentrations tested, it was not possible to determine the MIC. Positive controls: streptomycin (STR) and chloramphenicol (CHL)

Table 3. Radical scavenging ability of essential oils and ethanolic extracts from *Lippia turbinata, Clinopodium gilliesii, Lippia integrifolia, Zuccagnia punctate*, and *Senecio subulatus* var. *salsus*.

	DPPH radical scavenging
Essential oils	**$SC^{\dagger}_{50}$ (µg/ml)**
L. turbinata	447.2 ± 7.7[f]
C. gilliesii	N/D
L. integrifolia	N/D
Z. punctata	N/D
S. subulatus	3429.6 ± 489.8[g]
Ethanolic extracts	
L. turbinata	57.39 ± 0.34[c]
C. gilliesii	65.14 ± 5.64[c]
L. integrifolia	159.33 ± 9.43[d]
Z. punctata	18.83 ± 0.21[b]
S. subulatus	255.80 ± 4.71[e]
Controls	
BHT	55.5 ± 3.9[c]
Ascorbic acid	4.1 ± 0.4[a]

†SC: concentration required to scavenge 50% DPPH radicals. ND: not determined. In the range of concentrations tested we were unable to determine SC_{50}. BHT: butylated hydroxytoluene. Values are means ± SD. Means in a column without a common letter differ (p<0.05).

dant (Table 3). *L. turbinata* and *C. gilliesii* EEs also showed good radical scavenging activity in DPPH assay, in fact the SC_{50} values of these extracts were similar to those of BHT (Table 3). The order of antioxidant capacity for the EEs analyzed was as follows: *Z. punctata* > *L. turbinata* = *C. gilliesii* > *L. integrifolia* > *S. subulatus* var. *salsus* (Table 3).

The radical scavenging capacity of three flavonoids isolated from *Z. punctata* was demonstrated and it has been proposed that they would be the bioactive compounds of the plant (Morán Vieyra et al. 2009). Taking into account the above mentioned and the high amounts of flavonoids in our *Z. punctata* EE (approximately 64% of the total phenolic content), it is very likely that these compounds are responsible for the strong antioxidant activity observed.

On the other hand, the antioxidant activity of *C. gilliesii* EE was similar to that reported by Cabana et al. (2013) and higher to that reported by Reynoso et al. (2018). In the case of *L. turbinata,* there are no reports on the biological activity of its EE so our results constitute the first report of its antioxidant capacity.

The EEs showed a better antioxidant capacity than EOs, thereby indicating that antioxidant activity would be concentrated in the non-volatile fraction of the plants. Polyphenols are recognized as important antioxidant compounds and the correlation between them and radical scavenging activity in plant extracts have been reported (Dai and Mumper 2010; Grzegorczyk-Karolak and Kiss 2018; Dirar et al. 2019). In our study, correlation analysis for SC_{50} values and total phenolics in the extracts indicated a significant inverse relationship (r = -0.85; p < 0.01), which means a good correlation between these parameters. Thereby, extracts with low SC_{50} values also have high phenolic content. Furthermore, a moderate inverse correlation was observed between the SC_{50} values and total flavonoid content in the extracts (r=-0.55; p<0.05).

Thus, the antioxidant activity of studied plants could be attributed mainly to the phenolic compounds present in non-volatile fraction.

EOs and EEs anti-inflammatory properties

It is known the relationship between reactive oxygen species (ROS) and reactive nitrogen species (RNS) with inflammatory response. Thus, plant products that have antioxidant activity on many occasions also have anti-inflammatory effect (Miguel 2010; de Lavor et al. 2018). Keeping this in mind, we evaluated the inhibitory effect of our plant products on a pro-inflammatory enzyme, the cyclooxygenase 2 (COX-2). Inhibition of COX activity is the mechanism by which non-steroidal anti-inflammatory drugs exert their therapeutic effects (Ikeda et al. 2019).

Figure 2 shows the inhibition of COX-2 activity by the five EOs and EEs studied. In the COX-2 essay the oils were tested at 150 and 300 µg/ml while the extracts were evaluated at lower concentrations than the oils (50 and 150 µg/ml) since they showed higher antioxidant activity.

Among the five EOs, *C. gilliesii* presented the highest anti-inflammatory activity. In fact, this EO reached 46 and 60% of COX-2 inhibition at 150 and 300 µg/ml, respectively. A significant decrease in COX-2 activity (54% of inhibition) was also observed with *L. integrifolia* EO followed by *L. turbinata* EO, *S. subulatus* var. *salsus* EO, and *Z. punctata* EO at 300 µg/ml (Figure 2A).

Reynoso et al. (2018) have demonstrated the anti-inflammatory capacity of *C. gilliesii* EO in two rat models: carrageenan induced plantar edema (acute inflammation) and granuloma formation by cotton disc (chronic inflammation). In agreement with that, our results showed that *C. gilliesii* EO was active on pro-inflammatory enzyme COX-2. Since oxygenated monoterpenes are the main components of *C. gilliesii* EO, the inhibitory effect on

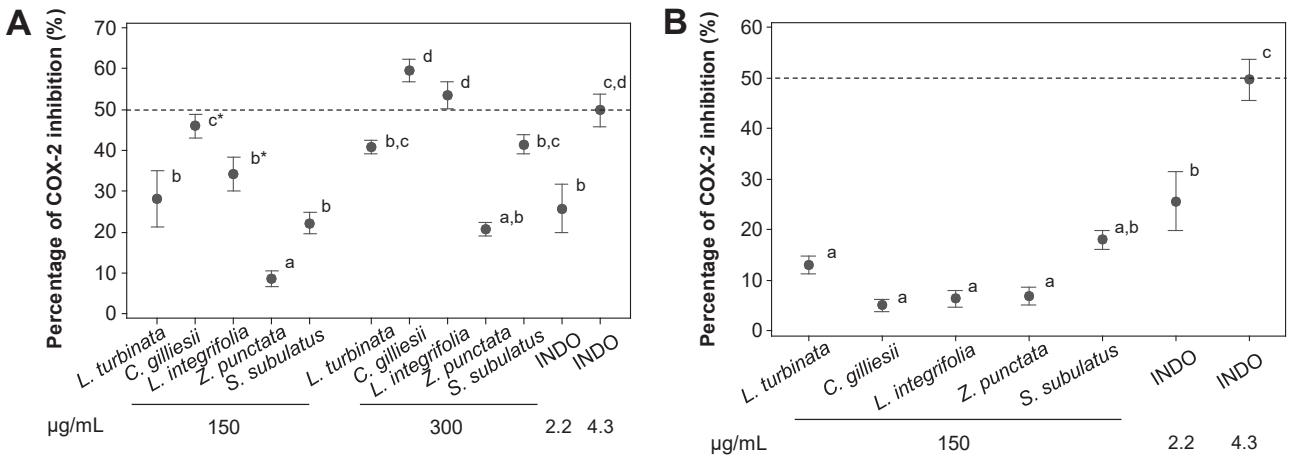

Figure 2. Effect of essential oils (A), ethanolic extracts (B) and indomethacin (INDO, A, B) on COX-2 enzyme activity. Data are the mean ± SD. Means without a common letter (a, b, c, d) differ, $p<0.05$. *Significantly higher than its corresponding extract at the same concentration (150 µg/ml), $p<0.05$

COX-2 could be attributed to them. In this sense, several oxygenated monoterpenes such as pulegone, menthone and piperitenone oxide have been reported to have anti-inflammatory activity (De Cássia da Silveira e Sá et al. 2013).

In the case of *L. integrifolia* EO the inhibitory activity on COX-2 would be related to the presence of oxygenated sesquiterpenes like *trans*-davanone and spathulenol, main constituents of the EO.

Considering the good antioxidant activity showed by the EEs, we expected to find higher inhibitory activity against COX-2 enzyme. However, the extracts showed no inhibition activity at 50 and at 150 µg/ml the inhibition percentages were not more than 20% (Figure 2B). Compared to EOs the EEs were poor COX-2 inhibitors. *C. gilliesii* and *L. integrifolia* EOs were 9-fold and 5-fold more potent on COX-2 compared to their corresponding EEs at the same concentration (150 µg/ml) (Figure 2).

CONCLUSION

The vegetal species as well as the extracts (EO and EE) studied showed differences in their phytochemical and biological activity. Thus, our results showed that the *L. turbinata*, *C. gilliesii,* and *Z. punctata* EE have a strong antioxidant activity and it is higher than the activity of their corresponding EOs. In addition, *C. gilliesii* and *L. integrifolia* EOs were active against the inflammatory COX-2 enzyme and this inhibitory activity was more potent in the EOs than EEs. This would indicate that antioxidant activity is concentrated in the non-volatile fraction of the plants whether the anti-inflammatory activity is in the volatile one.

This work contributes to knowledge of biological properties of plants from our region and could help to discover compounds with potential therapeutic uses, as food preservatives or anti-inflammatory agents.

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AUTHORS' CONTRIBUTIONS

NB: experimental studies, acquisition and analysis of data, manuscript preparation. MG: acquisition and analysis of data, manuscript preparation. AB: manuscript editing and review. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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