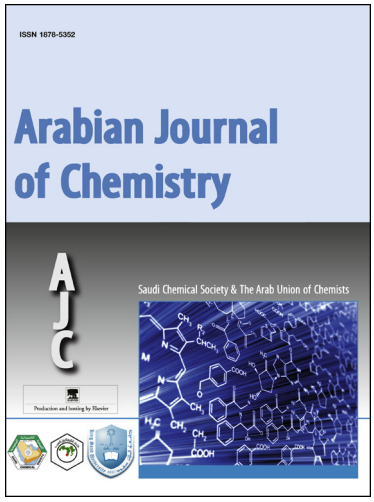

Original article

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Chemical composition, antibacterial and repellent activities of Azorella trifurcata, Senecio pogonias, and Senecio oreophyton essential oils.

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§equal contribution

Dedicated to the memory of Ing. Francisco Benavente (Facultad de Ingeniería, Universidad Nacional de San Juan, Argentina)

Abstract

The antibacterial and insect-repellent activities of the essential oils (EOs) from Argentinian medicinal plants *Azorella trifurcata* (Gaertn.) Pers., *Senecio cfr. oreophyton* J. Remy and *Senecio cfr. pogonias* Cabrera, were investigated. All EOs showed good repellent properties against *Triatoma infestans* Klug, the vector of the Chagas disease, with percent repellence values between 60% and 70% at 24 hours compared with positive control N-N diethyl-m-methylbenzamide (DEET) and moderate activity against the bacteria tested with minimum inhibitory concentrations (MICs) values between 31.2 and 2000 μg/ml. The *Azorella trifurcata*, *Senecio pogonias* and *S. oreophyton* EOs, obtained by hydrodistillation, were characterized by GC-FID and GC/MS analyses. Spathulenol (38.2%), myrtenyl acetate (8.4%) and α-terpineol (4.5%), limonene (9.8%) and α-thujene (5.4%) were the main constituents in the EO of *Azorella trifurcata*. The *Senecio pogonias* and *S. oreophyton* EOs are characterized by a high content of monoterpenes hydrocarbons (92% and 95.1%, respectively) with α-pinene, the main component in both oils. To our knowledge, the essential oil composition from Andean medicinal plants *A. trifurcata*, *Senecio pogonias* and *S. oreophyton* collected in central Andean slopes are reported for first time.

Keywords: *Azorella trifurcata*, *Senecio oreophyton*, *S. pogonias*, essential oil, *Triatoma infestans*, Chagas disease, *Escherichia coli*.

1. Introduction

The province of San Juan is located in the central-western part of Argentina, centred on the intersection of 31° S latitude and 69 ° W longitudes to the western Andean slopes. The province has a rich tradition in folk medicine including the use of medicinal plants. The flora from ecosystem Andino in Argentine comprises a large number of species distributed in different ecosystems, characterized by particular edaphic and climatic conditions. Plants

of the high mountains have been used as medicines since pre-hispanic times and are still used for their reputed therapeutic properties, including *Azorella*, *Senecio*, *Baccharis* and *Larrea* genus, to treat mainly digestive and hepatic disorders, fever, coughs and colds (Bustos et al ., 1996; Feresin et al., 2002).

The *Azorella* genus comprises about 30 species growing in the Andean mountains and Patagonia, Argentina, and only 15 species are recognized in this country (Martinez, 1989) . Several authors have reported the chemical composition of the Chilean species *A. madreporica* and *A. yareta*, and *A. compacta* (Loyola et al., 1997a; Loyola et al ., 1997b; Loyola et al.,1997c; Loyola et al., 1998a; Loyola et al., 1998b; Loyola et al., 2002). The biological activities as: antiplasmodial, trichonomicidal, antituberculosis and antibacterial, have been evaluated of azorellane and mulinane diterpenoids, isolated of the different *Azorella* species Wächter et al., 1999; Loyola et al., 2001b; Loyola et al., 2004; Molina-Salinas et al., 2010). *Azorella trifurcata*, commonly known as "yareta" distributed in Argentina and Chile and is used to treat digestive disorders. Recently, triterpenoids isolated from *Azorella trifurcata* (Gaertn.) Pers and their effect against the enzyme acetylcholinesterase were reported (Areche et al., 2010). Also, their in vitro spermatostatic activity of mulinane- and azorellane-type diterpenes on human spermatozoa (Chiaramello et al., 2011) was reported.

Regarding to *Senecio* genus, there are about 3000 species around the world, mainly in hilly areas. In Argentina there are more than 270 species, most of them in the Andes Mountain and in Patagonia (Cabrera, 1971). The chemical composition of the essential oils of some *Senecio* species included *S. trapezuntinus, S. platyphyllus, S. vernalis, S. glaucus, S. leucostachys, S. squalidus, S. aegyptius, S. graveolens, S farfarifolius, S. nutans, S. rufinervis*, and *S. longipenicillatus* are been reported (Kahriman et al., 2011; Mishra et al., 2011). The species, *Senecio cfr pogonias,* and *S. cfr oreophyton* n.v. "chachacoma" are

used in traditional medicine of Argentine to treat hepatic disorders, fever, coughs and colds. To our knowledge, so far, the biological activities and chemical composition of A*zorella trifurcata*, *Senecio oreophyton* and *Senecio pogonias* essential oils (EOs) collected in central Andean zone of Argentina has not yet been reported.

Is recognized, the infectious diseases caused by bacteria, virus, fungi, and parasites, are still a significant threat to public health (WHO, 2012). Thus, the natural products, specially the EOs and their components, are an alternative by this type of affections.

We report the chemical composition, repellente against *Triatoma infestans* "Chagas disease" vector and antimicrobial activity of essential oils of *Azorella trifurcata*, *Senecio oreophyton*, and *S pogonias* collected in the central Andean mountain from San Juan province, Argentine.

2. Materials and methods

2.1. Plant materials

Samples of *Azorella trifurcata* (Gaertn.) Pers. (Apiaceae), *Senecio cfr. oreophyton* J. Remy and *Senecio cfr. pogonias* Cabrera (Asteraceae) were collected from Iglesia (Quebrada de Romo) district in the Central Andes area, from San Juan province, Argentina, during the flowering period (2011). The species were identified by Dr. Luis Ariza Espinar (Instituto Multidisciplinario de Biología Vegetal, CONICET, and Universidad Nacional de Córdoba). Voucher specimens were deposited with the Museo Botánico de Córdoba, Argentina and were identified as CORD 9745, CORD 9748 and CORD 9747 code, respectively.

2.2. Isolation of essential oils

Fresh aerial parts (500 g) were finely grinded and subjected to hydro distillation in a Clevenger apparatus for 1 h, according to the method recommended by European

Pharmacopoeia (Council of Europe (COE, 2005). The yields were averaged over four distillations and calculated according to dry weight of the plant material. Essential oils (EOs) were stored at -18 ºC in airtight micro tubes prior to analysis by gas cromatography (GC) and chromatography-mass spectrometry (GC/MS).

2.2.1. Chemical characterization of the essential oils. GC Analysis

The GC analyses were performed with a Shimadzu GC-R1A apparatus equipped with a flame ionization detector (FID) and a DB-5 fused-silica cap. column (30 m x 0.25 mm i.d., film thickness 0.25 mm) coated with a non polar 5% phenyl/95% dimethylpolysiloxane phase. The oven temp. was programmed from 40 to 2308 at 28/min; injector and detector temp., 2408; carrier gas, N_2 (0.9 ml/min). The identification of the components was based on the comparison of their retention indices (RIs) with those of a homologous series of n-alkanes (C_9–C_{25}) and of pure authentic samples.

2.2.2. GC-MS Analysis of essential oils

The essential oils were analyzed by GC/MS. Mass spectra were obtained on a Perkin Elmer Clarus 600 mass spectrometer, coupled directly to Perkin Elmer Series Clarus 600 gas chromatograph fitted with a on a fused silica DB-5 MS capillary column (60 m, 0.25 mm i.d., film thickness 0.25 μm); using helium as a carrier gas (49.6 psi). The split injection mode was selected. Samples were analyzed at oven temperature program: initial temperature 60 ºC (held for 5 min), 5 ºC min to 240 ºC (held for 10 min). A column head pressure of 15 psi and an injector temperature and FID detector of 250 ºC were used. The GC transfer line was maintained at 200 ºC. Ionization was carried out in the mass spectrometer under vacuum by electron impact with a 70 eV ionization energy. Chromatograms were acquired in “scan” mode scanning the quadrupole from m/z 50 to m/z 300 (scan time: 0.2 s, inter-scan time: 0.1 s).

Identification of the components was done comparing their mass spectra with those reported in literature and by computer matching with the Wiley 8 and Adams libraries and co-injection with authentic compounds whenever was possible (Adams, 2007).

2.3. Repellent activity against Triatoma infestans nymphs

2.3.1. Insects

Triatoma infestans Klug nymphs were provided by Servicio Nacional de Chagas (Córdoba, Argentina) at the fifth instar. Nymphs were used one day after receipt.

2.3.2. Repellent activity test

Bioassays were done according to Talukder and Howse (Talukder and Howse, 1994). Filter paper discs (9 cm diameter) divided by halves were used. One half was treated with 0.5 ml of acetone solutions of the essential oils (0.5% w/v) remaining the other half untreated. As control, circular white filter papers divided in two halves, one treated with 0.5 ml of acetone and the other untreated, were used. After solvent evaporation, filter paper discs were placed covering the floor of a Petri dish. Five starved nymphs of *T. infestans* (fifth instar) were released in the centre of each Petri dish and maintained under controlled conditions of temperature 24 ± 2 ºC, 50 ± 5% RH and photoperiod of 16 h L/8 h D. Experiments were performed by quintuplicate. The insect's distribution was recorded at 1, 24 and 72 h of treatment. Data were transformed into repellency percentage (RP) as:

RP = (Nc-50) x 2

Where Nc represents percentage of nymphs on the blank half of the filter-paper disk.

Positive values show repellence while negative values show attraction. Mean values were categorised according to the following scale: Class 0 (> 0.01 to < 0.1), I (0.1 to 20), II (20.1 to 40); III (40.1 to 60); IV (60.1 to 80), V (80.1 to 100). Data were analyzed by repeated measures ANOVA to determine the overall significance of the repellence means

between the time points and the effect of oil treatment as a factor between subjects. Data were analysed with the statistical software SPSS 15.0 (SPPSS Inc.).

2.4. Antibacterial activity

2.4.1. Microorganisms

The following bacterial strains were used: *Escherichia coli* ATCC 25922, *Escherichia coli* LM1 (LM: Laboratorio de Microbiología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina). *Escherichia coli* 712, *E- coli* 121, *E- coli* 782, *Enterobacter* sp., *Pseudomonas* sp., *Klebsiella pneumoniae* ss. *pneumoniae*, *Klebsiella pneumoniae* ss. *pneumoniae* 94-1, *Staphylococcus coagulasa negative*, and *Proteus mirabilis* (Laboratorio de Microbiología, Hospital Marcial Quiroga, San Juan, Argentina).

2.4.2. Antibacterial activity test

Minimum inhibitory concentration (MIC) was determined using the microbroth dilution method according to the protocols of the Clinical and Laboratory Standards Institute (CLSI, 2008). All tests were performed in Mueller-Hinton broth (MHB), and cultures of each strain were prepared overnight. Microorganism suspensions were adjusted in a spectrophotometer with sterile physiological solutions to give a final organism density of 0.5 Mc Farland scale (1-5 x 105 CFU/ml). Stock solutions of EOs in DMSO were diluted to give serial two-fold dilutions that were added to each medium to obtain final concentrations ranging from 2000-32.5 µg/ml. The final concentration of DMSO in the assay did not exceed 1%. The antimicrobial agent cefotaxime (Argentina Pharmaceutica) as a positive control. Tests were done in triplicate and values of MIC are expressed in µg/ml.

3. Results and discussion

Hydrodistillation of the fresh leaves and stems of *A. trifurcata*, *Senecio pogonias* and, *Senecio oreophyton* gave pale yellow and green essential oils in yields of 1.0%, 0.4%, and 1.0% (w/v), respectively. EO components and relative percentages analyzed by GC/FID and GC/MS of *A. trifurcata* with a total of 25 constituents that represented 98.2% of the total essential oil were showed (Table 1). The monoterpenes represented the main fraction of the essential oils accounting for 49.5%, characterized by a high percentage of oxygenated monoterpenes (28.5%) and monoterpene hydrocarbons (21%). Spathulenol (38.2%), myrtenyl acetate (8.4%) and α-terpineol (4.5%), limonene (9.8%), and α-thujene (5.4%) were the main compounds identified, while that the hydrocarbon sesquiterpenes accounted for 10.5 %. Among them, the most abundant was α- guaiene (7.5%) and β-caryophyllene (3.0 %).

Regarding to chemical composition essential oils both species of *Senecio* (Table 1), a total of 19 and 18 compounds amounting 97.6% and 97.3% were identified in the EOs from *S. pogonias* and *S. oreophyton*, respectively. The EOs are characterized by a high content of monoterpenes hydrocarbons (92% and 95.1%) in *S. pogonias*, and *S. oreophyton*, respectively, being α-pinene, the the main component in both oils. Besides the essential oil of *S. pogonias* was characterized by other main component as α- phelandrene (22.0%), p-cymene (7.1%) and β-pinene (5.9%), while that *S. oreophyton* essential oil contain p-mentha-1(7), 8-diene (31%), and β- phelandrene (5.3%) as other major constituents.

3.1. Repellent activity of essential oils on T. infestans nymphs

Repellents are substances that act locally or at adistance, deterring an arthropod from flying to, landing on or bitinghuman or animal skin (or a surface in general) (Blackwell et al., 2003; Choochote et al., 2007).

The repellent activity at 1, 24 and 72 h after treatment against *Triatoma infestans* nymphs of *Azorella trifurcata*, *Senecio oreophyton* and *S. pogonias* EOs from Central Andes Argentina, are summarized in Table 2. According to the repeated measures ANOVA with a Greenhouse-Geisser correction, the repellent percentage differed significantly between time points ($p < 0.05$). Significant differences were observed between the oil treatment and control (effects between-subjects, $P < 0.05$). *A. trifurcata* and *S. oreophyton* EOs were found to be Class III repellents, while the *S. pogonias* showed a high repellency (class IV).

Regarding to the repellence percentage (RP) after 72-h treatment, the most repellent EOs were *S. pogonias* and *A. trifurcata* (68% RP). On the other hand, *S. oreophyton* essential oil repellency showed a peak within 24 hours (68%) however, at 72 h this value declined (36%).

Recently, the repellent activity of EOs of *G. polycephalum* and *A. cryptantha* (Apiaceae), from Argentinian Central Andes was reported on nymphs of *T. infestans* (Lima et al., 2011; Lopez et al., 2012).

A. trifurcata, *S. pogonias* and *S. oreophyton* EOs repellent activity may be due to the presence of terpenes as spathulenol, limonene, α-pinene, α–phellandrene, and p-cymene because of their recognized insect repellent properties. In bite deterrent studies, spathulenol, intermedeol, and callicarpenal showed significant repellent activity against *Aedes aegypti* and *Anopheles stephensi* (Cantrell et al., 2005). In a previous report on aromatic plants collected in central west of Argentina, (Gillij et al., 2008) suggest that limonene and camphor are the main components responsible for the repellent effect against *A. aegypti*. In addition, some monoterpenes such as α-pinene, cineole, eugenol, limonene, terpinolene, citronellol, citronellal, camphor, and thymol are common constituents of a numerous EOs described in the literature, as presenting mosquito repellent activity (Ibrahim and Zaki, 1998; Yang et al., 2004; Park et al., 2005; Jaenson et al., 2006). Comparisons of larvicidal data revealed that α-phellandrene, limonene, p-cymene, c-terpinene, terpinolene, and a-terpinene examined in this

study exhibited great larvicidal performance against *A. aegypti* and *A. albopictus* (Cheng et al., 2009).

However, the bioactivity of the EO depends upon the type and nature of their constituents and individual concentration. It further varies with species, season, location, climate, and soil type, age of the leaves, fertility regime, the method used for drying the plant material, and the method of oil extraction (Brooker and Kleinig, 2006). EOs from *A. trifurcata*, *S. pogonias,* and *S. oreophyton* collected in Argentinian Central Andean may be potential alternative repellents to *Triatoma infestans* (Klug) (Hemiptera, Reduviidae), the vector of Chagas disease, since they constitute a rich source of bioactive compounds that are biodegradable into non toxic products.

3.2. Antibacterial activity of essential oils

The antibacterial activity of the essential oils was evaluated against Gram-positive bacteria, Gram-negative bacteria. Their activity potential was assessed qualitatively by minimum inhibitory concentration (MIC) values. The antibacterial activity of EOs from argentinian medicinal plants *A. trifurcata, S. pogonias*, and *S. oreophyton* are showed in Table 3. From them, the EO of *A. trifurcata* was the most active against *Pseudomona* sp. *Staphylococcus coagulase negative* 968 with MICs values of 500 µg/ml and 1000 µg/ml respectively. EOs from *A. trifurcata*, *S. pogonias*, and *S. oreophyton* showed antibacterial activity towards enterobacterium *E. coli* ATCC 25922 and *E. coli*-LM-1 clinical isolate with a equal MIC values of 2000 µg/ml, being less active than standars antibiotics. Recently, the antimicrobial activity of *Azorella cryptantha* essential oil collected argentinian Andean montains was reported (Lima et al., 2011). The MICs values for Gram-negative bacteria reported in the present work were similar to those obtained for other plant species traditionally used to treat ailments related to bacterial infections in the

andean mountains of Argentina and colected between 2700 and 4800 m a.s.l. (Zampini et al.; 2009 López et al., 2012).

Food-borne illnesses associated with Gram (+) and Gram (-) bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enteritidis*, *Listeria monocytogenes* and *Bacillus cereus* present a major public health concern throughout the world.

The antimicrobial activity, against Gram negative and Gram positive bacteria of several *Senecio* spp. essential oil, including *Senecio graveolens*, *S. aegyptius*, *S. subpanduratus Senecio mustersii, S. pandurifolius S. atacamensis* were reported (Perez et al., 1999; El-Shazly et al., 2002; Arancibia et al., 2010; Benites et al., 2011; Kahriman et al., 2011). Components such as α-pinene, 1,8-cineole, γ-terpinene, linalool and α-terpineol have been found to have relatively strong antimicrobial properties (Bakkali et al., 2008). The EOs of the species *Azorella trifurcata, Senecio oreophyton*, and *S. pogonias* (Asteraceae) growing in locations in the central Andes (Argentina) showed a good antibacterial activity and support its medicinal uses.

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Table 1 Chemical composition of *A. trifurcata*, *Senecio pogonias* and *Senecio oreophyton* essential oils.

RI [a]	Compound	Composition (%) [b]			Identification [c]
		A. trifurcata	*S. pogonias*	*S. oreophyton*	
931	*α-thujene*	5.4	tr	0.6	
939	*α-pinene*	-----	48.0	40.0	Co
950	*α-fenchene*	1.2	tr	tr	
953	*camphene*	-----	-----	tr	
974	*sabinene*	0.4	1.7	2.2	
980	*β-pinene*	0.5	5.9	4.8	Co
990	*β-myrcene*	0.6	2.7	2.6	Co
992	*2,3-dehydro-1,8-cineole*	-----	0.4	tr	
1003	*α-phellandrene*	1.3	22.0	1.7	
1004	*p-mentha-1(7),8-diene*	-----	0.5	31.0	
1015	*α-terpinene*	-----	1.1	0.4	
1026	*p-cymene*	1.8	7.1	1.8	Co
1028	*o-cymene*	-----	-----	2.4	Co
1030	*limonene*	9.8	0.9	1.8	Co
1031	*β-phellandrene*	tr	1.0	5.3	
1033	*1,8-cineole*	2.0	1.3	0.3	Co
1057	*o-cresol*	2.3	-----	-----	Co
1062	*γ-terpinene*	-----	0.6	tr	
1090	*terpinolene*	-----	0.5	0.5	Co
1124	*4-terpinenyl acetate*	-----	tr	----	
1124	*α-campholenal*	0.2	tr	tr	
1138	*trans-pinocarveol*	0.4	0.3	tr	
1140	*cis-verbenol*	0.6	tr	tr	
1145	*camphor*	0.7	tr	-----	Co
1161	*trans-3-pinanone*	-----	tr	-----	
1166	*pinocarvone*	-----	tr	-----	
1171	*borneol*	0.3	-----	-----	Co
1178	*4-terpineol*	1.2	0.4	0.5	Co
1187	*cryptone*	0.2	-----	-----	
1191	*α-terpineol*	4.5	1.4	0.4	Co
1197	*myrtenol*	tr	-----	-----	
1225	*β-citronellol*	3.3	-----	-----	
1230	*cis-carveol*	-----	0.4	tr	
1254	*piperitone*	-----	1.4	0.5	
1255	*cis-geraniol*	2.0	-----	-----	Co
1290	*thymol*	2.4	-----	-----	Co
1330	*myrtenyl acetate*	8.4	-----	-----	
1390	*β-elemene*	-----	tr	tr	
1420	*β-caryophyllene*	3.0	tr	0.5	
1442	*α-guaiene*	7.5	-----	-----	
1478	*spathulenol*	38.2	-----	-----	
Total		98.2	97.6	97.3	
Monoterpene hydrocarbons		21.0	92	95.1	
Oxygenated monoterpenes		28.5	5.6	1.7	
Sesquiterpene hydrocarbons		10.5	0	0.5	
Oxygenated sesquiterpenes		38.2	0	0	

[a]) *RI*: Experimental Retention indices relative to homologous series of n-alkanes; [b]) Percentages were calculated from the peak area without correction; [c]) Mode of identification: Co: co injection authentic compounds; tr: traces.

Table 2 Repellent activity of essential oils from *Azorella trifurcata*, *Senecio pogonias, and Senecio oreophyton* against nymphs of *Triatoma infestans*, the vector of Chagas disease (Mean ± SEM, n=5).

	Repellency (%) at 0.5 % (w/v)[a]			Average RP[b]	Class[c]
	1h	24h	72h		
A. trifurcata	20.00 ± 25.30	76.00 ± 9.8	68.00 ± 19.60	54	III
S. pogonias	76.00 ± 16.00	60 ± 21.91	68 ± 14.97	68	IV
S. oreophyton	36.00 ± 9.80	68.00 ± 8.00	36.00 ± 14.97	46.6	III
DEET[d]	100 ± 0.0	100 ± 0.0	100 ± 0.0	100	V

[a]) Repellence percentage at 0.5 % [w/v] expressed as Mean ± SEM; [b]) Mean values of repellence percentage (RP) were categorised according to the following scale: [c]) Class 0 (>0.01 - <0.1 RP); Class I (0.1 – 20 RP); Class II (20.1 – 40 RP); Class III (40.1 – 60 RP); Class IV (60.1 – 80 RP); Class V(80.1 – 100 RP); [d]) Positive control (0.5 % w/v).

Table 3 Antibacterial activity of *Azorella trifurcata*, *Senecio pogonias*, and *Senecio oreophyton* essential oils. Value of MIC expressed in μg/ml.

Bacteria	MIC of essential oils			
	A. trifurcata	*S. pogonias*	*S. oreophyton*	cefotaxime
Escherichia coli ATCC 25922	2000	2000	2000	0.5
Escherichia coli-LM_1	2000	2000	2000	5
Escherichia coli 712	>2000	>2000	>2000	0.05
Escherichia coli 121	>2000	>2000	1500	1
Escherichia coli 782	>2000	>2000	>2000	1
Enterobacter sp	>2000	>2000	>2000	0.01
Pseudomona sp	500	>2000	>2000	2.5
Klebsiella pneumoniae ss. pneumoniae	>2000	>2000	>2000	>12
Staphylococcus, coagulasa negativa 968	1000	>2000	1000	5
Klebsiella pneumoniae ss. pneumoniae 94-1	>2000	>2000	>2000	>12
Proteus mirabilis 94-2	>2000	>2000	>2000	0.05

MIC: Minimum inhibitory concentration.