Essential Oil of *Azorella cryptantha* Collected in Two Different Locations from San Juan Province, Argentina: Chemical Variability and Anti-Insect and Antimicrobial Activities

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The essential oils (EOs) of two populations of *Azorella cryptantha* (Clos) Reiche, a native species from San Juan Province, were obtained by hydrodistillation in a *Clevenger*-type apparatus and characterized by GC-FID and GC/MS analyses. The compounds identified amounted to 92.3 and 88.7% of the total oil composition for *A. cryptantha* from Bauchaceta (*Ac-BAU*) and Agua Negra (*Ac-AN*), respectively. The EO composition for the two populations was similar, although with differences in the identity and content of the main compounds and also in the identity of minor components. The main compounds of the *Ac-BAU* EO were α-pinene, α-thujene, sabinene, δ-cadinene, δ-cadinol, *trans*-β-guaiene, and τ-muurolol, while α-pinene, α-thujene, β-pinene, γ-cadinene, τ-cadinol, δ-cadinene, τ-muurolol, and a not identified compound were the main constituents of the *Ac-AN* EO, which also contained 3.0% of oxygenated monoterpenes. The repellent activity on *Triatoma infestans* nymphs was 100 and 92% for the *Ac-AN* and *Ac-BAU* EOs, respectively. Regarding the toxic effects on *Ceratitis capitata*, the EOs were very active with LD_{50} values lower than 11 µg/fly. The dermatophytes *Microsporum gypseum*, *Trichophyton rubrum*, and *T. mentagrophytes* and the bacterial strains *Escherichia coli* LM_1, *E. coli* LM_2, and *Yersinia enterocolitica* PI were more sensitive toward the *Ac-AN* EO (*MIC* 125 µg/ml) than toward the *Ac-BAU* EO. This is the first report on the composition of *A. cryptantha* EO and its anti-insect and antimicrobial properties.

Introduction. – The Iglesia district is in the northwestern part of the San Juan Province (S 30°11′0″, W 69°9′ 0″). This area in central-western Argentina is a generally dry zone that includes many of the highest mountains of South America. Some endemic species that have evolved in this ecoregion show adaptations to extremely dry, cold, and windy conditions. They frequently have spines as antiherbivore defenses and conspicuous flowers to attract pollinators.

The Apiaceae (Umbelliferae) is a cosmopolitan family with 480 botanical genera and 2600 species [1]. The *Azorella* Lam. genus comprises *ca.* 30 species growing in the Andean Mountains and Patagonia, Argentina, and only 15 species have been

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recognized in this country. *Azorella cryptantha* (CLOS) REICHE, ex *Mulinum cryptanthum*, grows in the Chilean and Argentinean Andes [2]. In San Juan Province, the whole plant decoction is used as a cholagogue, blood depurative, digestive, expectorant, and antigonorrheal [3]. This species is locally known as '*yerba del soldado*' or '*cuerno de cabra*'.

Azorella species are commonly known as '*yareta*' or '*llareta*'. Several authors have reported the chemical composition of the Chilean species *A. madreporica*, *A. yareta* [4–11], and *A. compacta* [12]. *Colloca et al.* [13] reported the isolation of the diterpenes of *A. cryptantha*. The chemical composition and biological activity of the essential oil (EO) have not been reported yet.

EOs are an effective alternative against insect pests that affect agriculture products and human health [14]. The Mediterranean fruit fly, *Ceratitis capitata* WIEDEMANN (Diptera, Teprhitidae) is a pest that attacks large crops worldwide [15]. Insect pests hinder the agricultural production directly through harm production and indirectly through legal regulations such as quarantine and monitory programs. The WHO [16] reported that in Central and South America, seven million people are infected by *Chagas* disease, whose vector is *Triatoma infestans* (KLUG) (Hemiptera, Reduviidae). San Juan is affected by both problems. A recent study showed that the EOs from six Andean species collected in San Juan might be used to treat fungal infections and to improve the local *Chagas* disease situation by vector control [17].

The aim of this study was to report on the variability of the volatile EO components and of the toxic, repellent, antifungal, and antibacterial activities of the species *A. cryptantha* growing in two locations of the Iglesia district in the central Andes area of San Juan Province.

Results and Discussion. – Samples of *A. cryptantha* were collected near Bauchaceta (*Ac-BAU*), where the species is known as '*yerba del soldado*', and in Agua Negra (*Ac-AN*), where the vernacular name is '*cuerno de cabra*' (*Fig. 1* and *Table 1*).

The *A. cryptantha* plants in Agua Negra grew as a woody cushion at an altitude of *ca.* 4000 m a.s.l. Cushion plants are characterized by a densely branched hemispherical or mat-like growth, but differ from true mats by the presence of a central taproot (*Fig. 2*). On the other hand, the *A. cryptantha* plants collected near Bauchaceta grew as a shrub at an altitude of *ca.* 2800 m a.s.l. (*Fig. 3*).

Chemical Composition of the Essential Oils. The EOs were obtained by hydrodistillation from the air-dried parts of the plants. *Table 1* shows the EO yields, the details concerning the collection sites, and the vernacular and voucher specimen names of the two *A. cryptantha* samples. The EO yields were 0.4 and 1.0% (*w*/*v*) for *Ac-AN* and *Ac-BAU*, respectively.

Table 2 shows the EO components and relative percentages obtained by GC-FID and GC/MS analysis. A total of 26 and 30 compounds, amounting to 92.3 and 88.7% of the total oil composition, were detected in the *Ac-BAU* and *Ac-AN* EOs, respectively. The chemical compositions of the two EOs were similar, with variations in the relative proportion of the main components (*Table 2* and *Fig. 4*). In the *Ac-BAU* EO, the dominant components in the hydrocarbon fraction (48.3 and 27% for mono- and sesquiterpene hydrocarbons, resp.) were the monoterpenes α-thujene, α-pinene, and sabinene and the sesquiterpenes δ-cadinene, *trans*-β-guaiene, δ-cadinol, and τ-

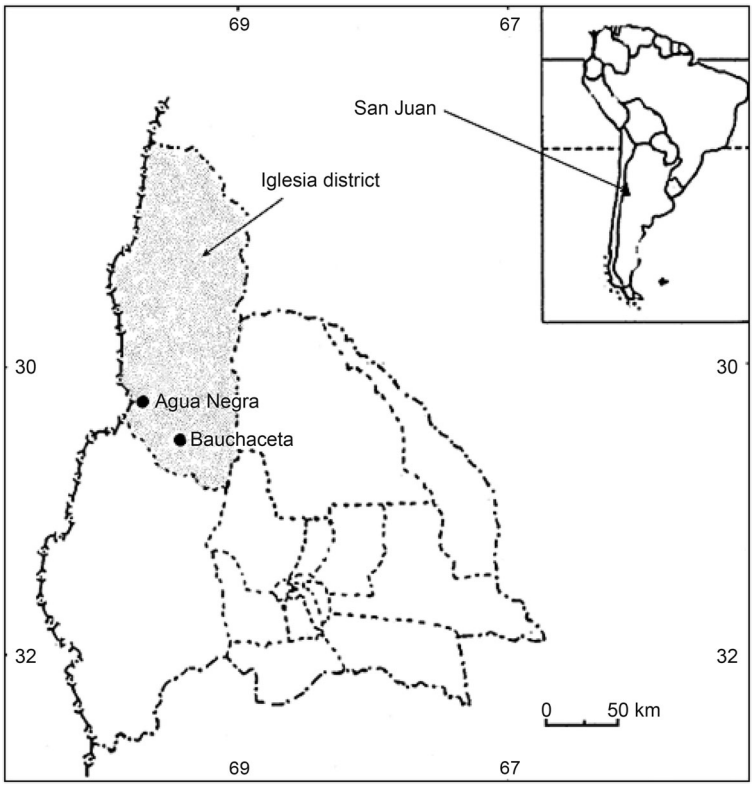

Fig. 1. *Collection areas of the* Azorella cryptantha *species in the Iglesia district of the San Juan province in Argentina: Agua Negra and Bauchaceta*

Table 1. *Abbreviation, Vernacular Name, Collection Site Details, Voucher Specimen Code, and Essential-Oil Yield of the* Azorella cryptantha *Species Collected in Bauchaceta and Agua Negra*

Abbreviation	Vernacular name	Collection site	Geographic coordinates	Altitude [m a.s.l.]	Voucher code	Yield [%][a])
Ac-BAU	'*Yerba del soldado*'	Bauchaceta	S 30°10′40″, W 69°27′30″	2800	CORD 1193	1.0
Ac-AN	'*Cuerno de cabra*'	Agua Negra	S 30°19′27″, W 69°12′39″	4000	CORD 1188	0.4

[a]) Essential-oil yield in % (*v*/*w*) based on the dry weight of the plant material.

muurolol. The *Ac-AN* EO had a lower content of monoterpene hydrocarbons (29.4%), represented mainly by α-thujene, α-pinene, and β-pinene. γ-Cadinene, τ-cadinol, τ-muurolol, and δ-cadinene were its main sesquiterpene hydrocarbon components. Only the *Ac-AN* EO contained oxygenated monoterpenes (3.0%; *cis*-β-terpineol, isoborneol, and borneol). The total content of sesquiterpene hydrocarbons was similar for

Fig. 2. Azorella cryptantha *collected in Agua Negra* (4000 m a.s.l.)

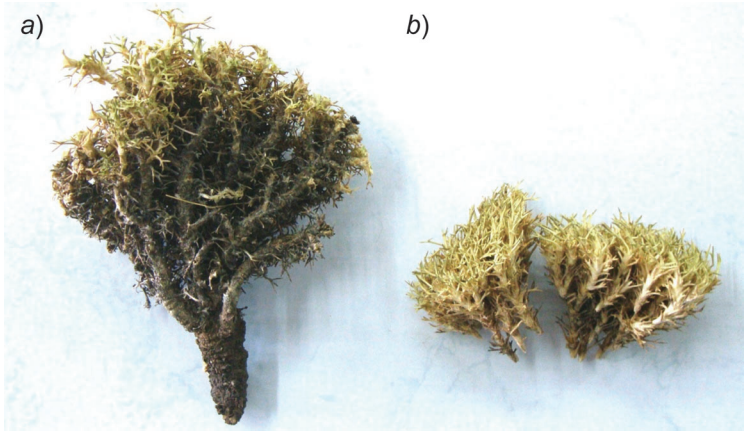

Fig. 3. *Characteristic* Azorella cryptantha *growth at different altitudes:* a) A. cryptantha *collected in Bauchaceta* (2800 m a.s.l.) *and* b) A. cryptantha *collected in Agua Negra* (4000 m a.s.l.)

both EOs, *i.e.*, 27.0 and 21.3% for *Ac-BAU* and *Ac-AN*, respectively, whereas the total content of the oxygenated sesquiterpenes was 17.0 and 12.4%, respectively.

To our knowledge, this is the first time that the EO composition of *A. cryptantha* has been reported. The results suggest that the main constituents are mainly the same for both populations, with some differences in minor components, like the oxygenated monoterpenes, for example.

Both populations are exposed to climatic conditions with extreme temperatures but at different altitudes. Our results regarding the chemical composition variability between the *A. cryptantha* EOs of the two populations agree with the study of *Alonso-Amelot* [18] concerning plants that are capable of growing successfully at high altitudes,

Table 2. *Chemical Composition of the Essential Oils Isolated from* Azorella cryptantha *Species Collected in Bauchaceta* (*Ac-BAU*) *and Agua Negra* (*Ac-AN*)

Compound name	*RI*[a])	Content [%][b])		Identification[c])
		Ac-BAU	*Ac-AN*	
α-Thujene	931	12.5	5.7	MS_1, Co
α-Pinene	940	21.9	9.6	MS_1, Co
Camphene	950	1.8	0.8	MS_2
Sabinene	974	6.4	–[d])	MS_2
β-Pinene	980	1.5	5.9	MS_1, Co
β-Myrcene	992	1.0	0.7	MS_2
α-Phellandrene	1011	–	0.1	MS_2
α-Terpinene	1018	–	1.0	MS_2
o-Cymene	1028	0.1	–	MS_2
Limonene[e])	1029	1.1	1.6	MS_2
β-Phellandrene	1035	0.6	2.5	MS_2
cis-β-Ocimene	1038	0.5	0.9	MS_2
trans-β-Ocimene	1051	0.5	0.5	MS_2
α-Terpinolene	1091	0.4	–	MS_2
cis-β-Terpineol	1145	–	0.8	MS_2
Isoborneol	1164	–	1.1	MS_2
Borneol	1170	–	1.1	MS_1, Co
α-Cubebene	1350	–	0.4	MS_2
α-Gurjunene	1409	–	0.4	MS_2
β-Caryophyllene	1418	1.9	1.1	MS_2
β-Gurjunene	1433	–	3.7	MS_2
γ-Gurjunene	1478	tr.[f])	0.7	MS_2
α-Humulene	1479	tr.	0.6	MS_2
Germacrene D	1487	–	0.2	MS_2
γ-Muurolene	1494	0.6	–	MS_2
α-Muurolene	1499	2.0	1.6	MS_2
α-Farnesene	1506	–	2.4	MS_2
γ-Cadinene	1512	3.6	4.0	MS_2
δ-Cadinene	1522	8.6	6.3	MS_2
α-Cadinene	1552	0.6	–	MS_2
α-Calacorene	1558	tr.	–	MS_2
trans-β-Guaiene	1592	6.2	–	MS_2
Viridiflorol	1596	–	2.5	MS_2
cis-β-Guaiene	1623	3.2	–	MS_2
τ-Cadinol[e])	1640	1.8	4.6	MS_2
τ-Muurolol[e])	1642	8.5	5.0	MS_2
Cubenol	1647	–	0.2	MS_2
δ-Cadinol	1719	6.7	–	MS_2
N.i.[g])	1789	–	22.7	
Total		92.3	88.7	
Monoterpene hydrocarbons		48.3	29.4	
Oxygenated monoterpenes		–	3.0	
Sesquiterpene hydrocarbons		27.0	21.3	
Oxygenated sesquiterpenes		17.0	12.4	

[a]) *RI:* Retention indices determined relative to *n*-alkanes (C_9–C_{25}) on a *DB-5 MS* column. [b]) Content expressed as percentage of the total essential-oil composition and calculated from the peak area without correction factors. [c]) Mode of identification: MS_1, mass spectra identical with those of pure reference compounds; MS_2, mass spectra identical with published data; Co, co-injection of authentic compounds. [d]) –: Not detected. [e]) Critical co-elutions on the *DB-5 MS* column were resolved by adding the second dimension polarity separation on the *Supelcowax-10* column. [f]) tr.: Trace ($< 0.1\%$). [g]) N.i.: Not identified compound; MS: 222 (5, M^+), 204 (30), 189 (10), 161 (100), 133 (20), 119 (50), 105 (70), 91 (70), 84 (50), 67 (40), 55 (60), 41 (90).

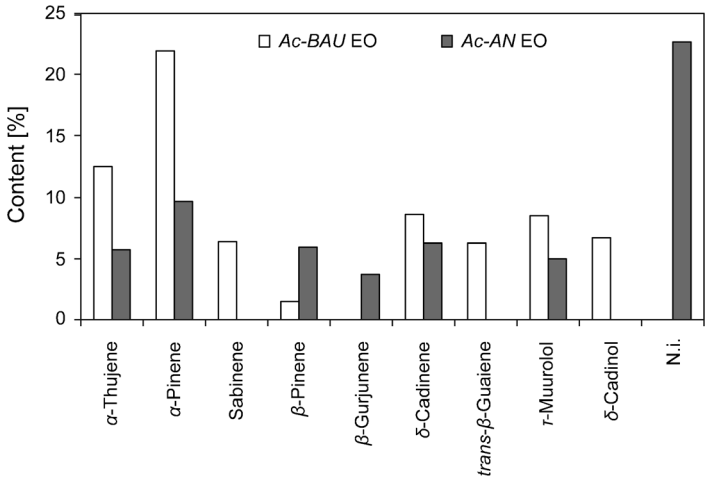

Fig. 4. *Content* [%] *of the main constituents of the essential oils isolated from* Azorella cryptantha *species collected in Bauchaceta* (*Ac-BAU*) *and Agua Negra* (*Ac-AN*)

due to highly specialized physiological processes that affect their chemical response. These reach from the synthesis of special lipids that modify cell membranes for flexibility and H_2O permeability to other features like the cushion-growth form of *A. compacta* as a way of reducing extreme temperatures and H_2O fluctuations, process not seen in low-altitude plants [19].

Interestingly, although the Apiaceae in South America are a renowned botanical family of herbs and spices, their EO compositions and biological activity against different pathogens (bacteria and fungi), pests, or vectors are currently unknown for most of the native species.

Insecticidal Activity of the Essential Oils against Ceratitis capitata *Adults after Topical Application.* The *A. cryptantha* EOs showed important insecticidal effects on the Mediterranean fruit fly (*C. capitata*) after the topical application of doses of 100, 50, and 10 μg EO/insect. The mortality was recorded at 24, 48, and 72 h after treatment. According to the repeated ANOVA with a *Greenhouse–Geisser* correction, the mean mortality of *C. capitata* adults differed significantly between the time points ($p < 0.0001$). The dose factor, nested within the oil treatment, showed a significant interaction with time ($p = 0.004$). At a dose of 10 μg/fly, 36 to 42% of females and 40 to 64% of males died within 24 h after treatment, whereas a dose of 50 μg/fly provoked > 77% mortality in both males and females treated with *Ac-BAU* or *Ac-AN* EOs. A dose of 100 μg/fly caused between 93 and 99% of mortality. The LD_{50} did not exceed 11 μg/fly at 72 h after treatment (*Table 3*). The LD_{50} values at 72 h were 2.60 and 9.54 μg/fly (*Ac-BAU*) and 10.78 and 8.39 μg/fly (*Ac-AN*) against males and females, respectively. The toxic properties of the oils were not significantly different against females, according to the confidence limits, while the EO from *Ac-BAU* had a significantly higher toxicity than that of *Ac-AN* against males (*Table 3*). *C. capitata* adults were susceptible to the positive control cypermethrin at < 3 ng/fly. The toxic activity of EOs from some species of *Tagetes* against both sexes of *C. capitata* adults have been reported

by us with LD_{50} values up to 20 μg/fly [14]. In comparison to these *Tagetes* EOs, the *A. cryptantha* EOs showed a stronger activity as toxicant agents against adults of the Mediterranean fruit fly.

Table 3. *Topical Application of the Essential Oils Isolated from* Azorella cryptantha *Species Collected in Bauchaceta* (*Ac-BAU*) *and Agua Negra* (*Ac-AN*) *and of the Positive Control Cypermethrin on Male and Female* Ceratitis capitata *Adults* ($n=30$)

Sample	Sex[a])	LD_{50}[b])	CL[c])	Slope ± SE[d])	χ^{2}[e])	*df*[f])
Ac-BAU	♂	2.60	0.18–6.21	1.21 ± 0.25	45.016	28
	♀	9.54	4.70–14.26	2.12 ± 0.26	81.56	28
Ac-AN	♂	10.78	8.27–13.27	2.36 ± 0.27	27.85	28
	♀	8.39	3.64–13.15	1.35 ± 0.20	40.60	28
Cypermethrin	♀	1.50	0.70–2.30	1.06 ± 0.12	37.90	28
	♂	2.43	0.90–5.40	0.76 ± 0.11	73.39	28

[a]) Sex of the *C. capitata* adults. [b]) LD_{50}: Lethal dose for 50% mortality after exposure for 72 h, expressed as [μg/insect] for the EOs and as [ng/insect] for cypermethrin. [c]) CL: 95% Confidence limits. [d]) Parameter estimates for the *Probit* model at 72 h. [e]) χ^2: Chi-square value. [f]) *df:* Degree of freedom.

The presence of monoterpene hydrocarbons like α-thujene and α- and β-pinene were found, by *Passino et al.* [20], to decrease the toxic activity of EOs in diet formulations for *C. capitata* larvae. Interestingly, these are the main components in the EOs of *A. cryptantha*. However, the role of minor components cannot be dismissed in the light of growing evidence of synergy among the components of EOs [21–23]. In addition, the presence of sesquiterpenes (hydrocarbons and oxygenated) like δ-cadinene, τ-cadinol, and τ-muurolol could contribute significantly to the toxic activity. *Teucrium laucocladum* (Lamiaceae) EO, which shows important amounts of sesquiterpenes such as 1,10-diepicubenol, cubenol, α-muurolol, τ-cadinol, and α-cadinol has shown a marked toxicity against *C. capitata*, *Musca domestica*, and *Culex pipiens* larvae [24].

Repellent Activity of the Essential Oils against Triatoma infestans *Nymphs. Table 4* summarizes the repellent activity of the two EOs that showed excellent repellent properties against *T. infestans* nymphs. According to the repeated ANOVA with a *Greenhouse–Geisser* correction, the repellent percentage did not change significantly between time points (no effect within subjects, $p>0.05$) nor was there a significant interaction between time points and the oil treatments ($p>0.05$). Significant differences in the average repellency were observed between the treatments and the control (effects between subjects, $p<0.0001$). In spite of variations in the chemical composition of the oils, the repellent activity against *T. infestans* nymphs of both EOs was the highest on the repellency classification scale, since both oils were found to be in *Class V*. The recorded average repellencies were 92 and 100% for *Ac-BAU* and *Ac-AN*, respectively.

When comparing the repellent activities against *T. infestans* of EOs isolated from other Argentinean Central Andes species [17], the *A. cryptantha* EOs from Bauchaceta and Agua Negra were the most active. One of the main control strategies for eliminating *Chagas* disease is based on controlling the transmission by domestic and peridomestic vectors. Thus, the particular compositions of the *A. cryptantha* EOs might contain promising chemical agents to control the vector of *Chagas* disease.

Table 4. *Repellent Activity of the Essential Oils Isolated from* Azorella cryptantha *Species Collected in Bauchaceta* (*Ac-BAU*) *and Agua Negra* (*Ac-AN*) *and of the Positive Control Tetramethrin against* Triatoma infestans *Nymphs*

Sample	Repellency [%][a])			Average repellency [%]	Class[b])
	1 h	24 h	72 h		
Control[c])	−12±76.9	−28±71.6	−100±0.0	−21.3 (a)[d])	–
Ac-BAU	76±21.9	100±0.0	100±0.0	92.0 (b)	*V*
Ac-AN	100±0.0	100±0.0	100±0.0	100.0 (b)	*V*
Tetramethrin[e]))	100±0.0	100±0.0	100±0.0	100.0 (b)	*V*

[a]) Repellence percentage (RP) at an essential-oil concentration of 0.5% (*w*/*v*) expressed as mean± SEM. Positive values show repellence, while negative values show attraction. [b]) Mean values of RP (average repellency) were categorized according to the following scale: *Class 0* (0.01–<0.1%), *Class I* (0.1–20%), *Class II* (20.1–40%), *Class III* (40.1–60%), *Class IV* (60.1–80%), and *Class V* (80.1–100%). [c]) Solvent control (acetone). [d]) Values followed by the same letter in parentheses within a column are not significantly different at the $p = 0.05$ level according to *Duncan*'s multiple-range test. [e]) Positive control at a concentration of 0.2% (*w*/*v*).

Antifungal Activity of the Essential Oils. Table 5 illustrates that all dermatophytes and yeasts tested were inhibited by the *Ac-AN* EO, which interestingly showed the lowest minimal inhibitory concentrations (*MIC*s of 125 μg/ml) against the dermatophytes assayed, suggesting the potential use of this species to treat dermatophytic infections. The *Ac-BAU* EO was less active against dermatophytes (*MIC* values between 500 and 1000 μg/ml). Regarding the yeast sensitivity, the *Ac-AN* EO was more active against *Cryptococcus neoformans* (*MIC* = 250 μg/ml) than against *Candida albicans*, *C. tropicalis*, and *Saccharomyces cerevisiae* (*MIC*s = 1000 μg/ml). The only yeast sensitive to the *Ac-BAU* EO was *C. neoformans* (*MIC* = 1000 μg/ml). On the other hand, the *Aspergillus* species were not sensitive to the *Ac-AN* EO, whereas the *Ac-BAU* EO was moderately active against the *A. niger* and *A. fumigatus* filamentous fungi with *MIC*s between 500 and 1000 μg/ml, respectively. Generally, the antimicrobial activity of an EO is considered as interesting when the *MIC* is lower than 1000 μg/ml [25]. On the other hand, the activity of the *Ac-AN* EO against the tested dermatophytes was only half that of thymol. This compound has been widely recognized as a fungicide and bactericide in previous work that includes studies of the mechanisms of action [26][27]. Briefly, the presented results indicate that the EOs analyzed were particularly active against dermatophyte strains and are clearly promising sources for treating superficial fungal infections.

Antibacterial Activity of the Essential Oils. The *Ac-AN* and *Ac-BAU* EOs showed inhibitory activities with *MIC*s between 125 and 1000 μg/ml against the tested bacteria (*Table 5*). The *Ac-AN* EO showed the better antibacterial activities than the *Ac-BAU* EO. The *Ac-AN* EO was active against the *Gram*-negative clinical isolates *E. coli* LM1, *E. coli* LM2, and *Yersinia enterocolitica* PI with *MIC* values of 125 μg/ml and against *Salmonella enteritidis* MI and *Salmonella* sp LM with *MIC* values of 250 μg/ml. The effect of both EOs was moderate against all the other bacteria tested (*MIC*s between 500 to 1000 μg/ml). A similar sensitivity of the *Gram*-negative bacteria than that to thymol was observed for the *Ac-AN* EO. *Areche et al.* [28] reported two new diterpenes isolated from *Azorella madreporica* with antimicrobial activity. Additionally, other

Table 5. *Antifungal and Antibacterial Activity of the Essential Oils Isolated from* Azorella cryptantha *Species Collected in Bauchaceta* (*Ac-BAU*) *and Agua Negra* (*Ac-AN*) *in Comparison to the Reference Compounds Thymol* (Thym), *Amphotericin B* (Amp B), *Ketoconazole* (Ket), *Terbinafine* (Terb), *and Cefotaxime* (Cef)

Microorganisms	Minimal inhibitory concentration [μg/ml]						
	Ac-BAU	*Ac-AN*	Thym	Amp B	Ket	Terb	Cef
Yeasts							
Candida albicans ATCC 10231	n.a.[a])	1000	125	1.00	0.50	–[b])	–
Candida tropicalis C 131 2000	n.a.	1000	125	0.50	0.12	–	–
Saccharomyces cerevisiae ATCC 9763	n.a.	1000	125	0.50	0.50	–	–
Cryptococcus neoformans ATCC 32264	1000	250	62.5	0.25	0.25	–	–
Filamentous Fungi							
Aspergillus flavus ATCC 9170	n.a.	n.a.	n.a.	0.50	0.12	–	–
Aspergillus fumigatus ATCC 26934	1000	n.a.	n.a.	0.50	0.25	–	–
Aspergillus niger ATCC 9029	500	n.a.	n.a.	0.50	0.50	–	–
Dermatophytes							
Microsporum gypseum C 115	1000	125	62.5	0.12	0.05	0.04	–
Trichophyton rubrum C 113	500	125	62.5	0.07	0.02	0.02	–
Trichophyton mentagrophytes ATCC 9972	500	125	62.5	0.07	0.02	0.04	–
Bacteria							
Staphylococcus aureus methicillin sensitive ATCC 29213	1000	750	250	–	–	–	0.5
Staphylococcus aureus methicillin resistant ATCC 43300	500	500	250	–	–	–	0.5
Escherichia coli ATCC 25922	1000	250	250	–	–	–	0.5
Escherichia coli LM_1	500	125	125	–	–	–	5.0
Escherichia coli LM_2	750	125	125	–	–	–	0.5
Pseudomonas aeruginosa ATCC 27853	750	500	500	–	–	–	7.5
Yersinia enterocolitica PI	500	125	125	–	–	–	0.5
Salmonella enteritidis MI	750	250	250	–	–	–	12.5
Salmonella sp LM	500	250	250	–	–	–	0.5

[a]) n.a.: Not active ($MIC > 1000$ μg/ml). [b]) –: Not determined.

authors [9][29–31] reported a trypanocidal effect of compounds isolated from species of the *Azorella* genus. This is the first report on the antimicrobial activity of EOs obtained from *Azorella* species growing in the Andes. *Bakkali et al.* [32] indicated the possibility that the activity of the main components might be modulated by another minor compound. Since EOs are complex mixtures of numerous molecules, their biological properties could be the result of a synergism of all molecules or only that of the main compounds present at the highest levels.

Conclusions. – The chemical composition and biological activity of *A. cryptantha* EOs from two different locations in the central Andes (Argentina) were analyzed. Their composition was similar, but with differences in the relative proportion of the main components and in the identity of minor components. The fact that only *Ac-AN* EO contained oxygenated monoterpenes could be interpreted as a response of the species to differences in altitude and climatic and edaphic conditions. Additionally, the *Ac-AN* EO was the most active against dermatophyte fungi. The reported activities

indicate that these EOs are promising to control pest insects and vectors and to treat dermatophyte-related infections.

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Experimental Part

Plant Material. In the February 2007 flowering period, samples of *Azorella cryptantha* (Clos) Reiche were collected near Bauchaceta, where the species is known as '*yerba del soldado*' (*Ac-BAU*), and in Agua Negra, where the vernacular name is '*cuerno de cabra*' (*Ac-AN*). Both Bauchaceta and Agua Negra are in the Iglesia district in the central Andes area, Province of San Juan, Argentina. The species was identified by *L. A. E.* (Instituto Multidiciplinario de Biología Vegetal, Universidad Nacional de Córdoba, Argentina), and voucher specimens have been deposited with the *Museo Botánico de Córdoba*, Argentina.

Isolation of Essential Oils. Fresh aerial parts (500 g) were subjected to hydrodistillation in a *Clevenger*-type apparatus for 1h, according to the method recommended by the *European Pharmacopoeia* [33]. The EO yields were averaged over four distillations and calculated as % (*v*/*w*) based on the dry weight of the plant material. The EOs were stored at $-18°$ in airtight microtubes prior to GC/MS analysis.

GC-FID and GC/MS Analysis. The GC-FID and GC/MS analyses of the EOs were performed with a *Perkin-Elmer Series Clarus 600* gas chromatograph equipped with a flame ionization detector (FID), a *Perkin-Elmer Clarus 600* mass spectrometer, and a *DB-5 MS* fused-silica cap. column (60 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temp. was programmed isothermal at 60° for 5 min, then rising from 60 to 240° at 5°/min, and isothermal at 240° for 10 min; injector and detector temp., 250°; transfer-line temp., 200°; carrier gas, He (49.6 psi); column head pressure, 15 psi. The split-injection mode was selected, and the ionization was carried out in the mass spectrometer under vacuum by electron impact (ionization energy, 70 eV). The chromatograms were acquired (scan mode, *m*/*z* 50–300) with a scan time of 0.2 s and an inter-scan time of 0.1 s.

The identification of the components was based on *i*) the comparison of their retention indices (*RI*s) determined rel. to the t_R values of a series of *n*-alkanes (C_9–C_{25}) with those reported in the literature [34], *ii*) the comparison of their mass spectra with those reported in the literature and by computer matching with the *Wiley 8* and *Adams* mass spectral libraries [34], and *iii*) coinjection with authentic compounds whenever it was possible.

Insects. Sterile males and females of *Ceratitis capitata* (*tsl* strain) were supplied by *ProCEM-SENASA* (San Juan, Argentina) at the pupae stage, two d before adults' emergence. Hatched adults were kept in chambers provided with H_2O *ad libitum* and artificial diet (sugar/yeast hydrolysate 3 : 1) under controlled conditions of temp. (24 ± 2°), relative humidity (50 ± 5%), and light (16 h L/8 h D). Sexes and cohorts were maintained separated. *Triatoma infestans* nymphs were provided by the *Servicio Nacional de Chagas* (Córdoba, Argentina) at the fifth instar. Nymphs were used one day after receipt.

Topical Application Bioassay on Ceratitis capitata *Adults.* The topical bioassay was carried out as described by *Siskos et al.* [35]. Randomly selected flies were narcotized by a stream of N_2 for a period of 5 min. The immobilized flies were picked up individually, and 2 μl of the test soln. was applied to the dorsum of each fly by means of an automatic micropipette. The doses used against both sexes were 100, 50, and 10 μg/insect. All doses were prepared from fresh stock solns. obtained by dissolving the EOs in acetone. Controls were run simultaneously and consisted of the same number of insects treated with 2 μl of acetone. The flies were 3–5 d old when tested. The mortality was recorded at 24, 48, and 72 h after treatment. The data were evaluated by repeated ANOVA, to determine the overall significance of the mortality means between the time points. Probit analysis was conducted on the mortality data recorded

after 72 h exposure to different doses of EO, to determine the lethal dose for 50% mortality (LD_{50}) for the respective sexes. Data were analyzed with the statistical software SPSS 15.0 (*SPPSS Inc.*).

Repellent Activity against Triatoma infestans *Nymphs.* The bioassays were carried out according to *Talukder* and *Howse* [36]. Filter paper discs (9 cm in diameter) divided into halves were used. One half was treated with 0.5 ml of a soln. of EO (0.5% (*w*/*v*) in acetone) and the remaining half was left untreated. Circular white filter papers divided in two halves, one treated with 0.5 ml of acetone and the other one untreated, were used as controls. After solvent evaporation, the filter-paper discs were placed in five *Petri* dishes. Five starved nymphs of *T. infestans* (fifth instar) were released in the center of each *Petri* dish and maintained under controlled conditions of temp. ($24\pm2°$), humidity ($50\pm5\%$), and a 16 h light/8 h darkness photoperiod. Experiments were performed in quintuplicate. The insect distribution was recorded at 1, 24, and 72 h of treatment. The data were transformed into repellence percentages (RPs) using *Eqn. 1:*

$$\text{RP}\,[\%] = (Nc - 50) \times 2 \tag{1}$$

where *Nc* represents the percentage of nymphs on the blank half of the filter-paper disk. Positive values show repellence, while negative values show attraction. The mean values of RP were categorized according to the following scale: *Class 0* (0.01 – < 0.1%), *Class I* (0.1 to 20%), *Class II* (20.1–40%), *Class III* (40.1–60%), *Class IV* (60.1–80%), and *Class V* (80.1–100%). The data were analyzed by repeated 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. *Post hoc* comparisons were carried out with *Duncan*'s multiple range test at $p = 0.05$. The data were analyzed with the SPSS 15.0 (*SPPSS Inc.*) statistical software.

Antimicrobial Activity. Microorganisms. The antibacterial activity of the EOs was assessed against the following bacterial strains: the methicillin-sensitive *Staphylococcus aureus* ATCC 29213 strain, the methicillin-resistant *Staphylococcus aureus* ATCC 43300 strain, *Escherichia coli* ATCC 25922, *Escherichia coli* LM_1 (LM = Laboratorio de Microbiología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina), *Escherichia coli* LM_2, *Pseudomonas aeruginosa* ATCC 27853, *Yersinia enterocolitica* PI (PI = *Pasteur Institute*), *Salmonella enteritidis* MI (MI = *Malbrán Institute*), and *Salmonella sp.* LM. The antifungal activity of the EOs was tested against the following fungal strains: *Candida albicans* ATCC 10231, *Candida tropicalis* C 131 (C = Centro de Referencia en Micología, FCByF, UNR), *Saccharomyces cerevisiae* ATCC 9763, *Cryptococcus neoformans* ATCC 32264, *Aspergillus flavus* ATCC 9170, *Aspergillus fumigatus* ATTC 26934, *Aspergillus niger* ATCC 9029, *Trichophyton rubrum* C 113, *Trichophyton mentagrophytes* ATCC 9972, and *Microsporum gypseum* C 115.

Antifungal Susceptibility Test. The minimum inhibitory concentrations (*MIC*s), defined as the lowest concentrations of oils that completely inhibit the visible growth of microorganisms in broth, were determined using the microbroth dilution method according to the protocols of the *National Committee for Clinical and Laboratory Standards* (NCCLS) [37][38], in 96-well microtiter plates with *RPMI-1640* (*Roswell Park Memorial Institute* medium, *Sigma*, St Louis, Mo, USA) broth at pH 7.0. The microtiter plates were incubated at 35° for yeasts and at 28–30° for dermatophyte strains. The inocula of cell or spore suspensions were obtained according to reported procedures [37][38] and adjusted to $1-5\times10^3$ cells/spores with colony forming units (CFU)/ml. Stock solns. of EOs in DMSO were diluted to give serial two-fold dilutions that were added to the medium at a final concentration of 0.98–256 μg/ml (100 μl final volume) and a final DMSO concentration $\leq 1\%$. Thymol, amphotericin B, ketoconazole, and terbinafine were used as positive controls.

Antibacterial Activity. The *MIC* values were determined using the microbroth dilution method according to the protocols of the NCCLS [39]. 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 soln. to give a final organism density of 0.5 *McFarland* scale ($1-5\times10^5$ CFU/ml). Stock solns. of EOs in DMSO were diluted to give serial two-fold dilutions that were added to each medium to obtain final concentrations ranging from 10–1000 μg/ml. The final concentration of DMSO in the assay did not exceed 1%. The antimicrobial agent Cefotaxime (*Argentia*

Pharmaceutica) and thymol were included in the assays as positive controls. The plates were incubated for 24 h at 37°. The *MIC* values were defined as the lowest EO concentrations showing no visible bacterial growth after the incubation time. Tests were done in triplicate.

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