Essential Oils of Medicinal Plants from the Central Andes of Argentina: Chemical Composition, and Antifungal, Antibacterial, and Insect-Repellent Activities

by **Beatriz Lima**[a]), **Sandra López**[a]), **Lorena Luna**[a]), **María B. Agüero**[a]), **Liliana Aragón**[a]), **Alejandro Tapia**[a]), **Susana Zacchino**[b]), **María L. López**[a]), **Julio Zygadlo**[c]), and **Gabriela E. Feresin***[a])

[a]) Instituto de Biotecnología – Instituto de Ciencias Básicas, Universidad Nacional de San Juan, Av. Libertador General San Martín 1109 (O), CP 5400, San Juan, Argentina (phone: +54-2644211700, int. 410/294; fax: +54-2644200289; e-mail: gferesin@unsj.edu.ar)

[b]) Área Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional del Rosario, Suipacha 531, CP 2000, Rosario, Argentina

[c]) Instituto Multidisciplinario de Biología Vegetal, Cátedra de Química Orgánica, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina

In memory of Dr. *Silvana Lucy Vallvé*

The antifungal, antibacterial, and insect-repellent activities of the essential oils (EOs) of *Acantholippia seriphioides*, *Artemisia mendozana*, *Gymnophyton polycephalum*, *Satureja parvifolia*, *Tagetes mendocina*, and *Lippia integrifolia*, collected in the Central Andes area, province of San Juan, Argentina, were investigated. The dermatophytes *Microsporum gypseum*, *Trichophyton mentagrophytes*, and *T. rubrum* were inhibited by the EOs of *G. polycephalum*, *L. integrifolia*, and *S. parvifolia*, with minimum inhibitory concentrations (*MIC*s) between 31.2 and 1000 μg/ml. Moreover, all EOs presented moderate activity against the bacteria tested, and the *L. integrifolia* and *G. polycephalum* EOs showed excellent repellent properties against *Triatoma infestans*, the *Chagas* disease vector, with repellency values between 60 and 100%. The *A. seriphioides*, *G. polycephalum*, and *L. integrifolia* EOs, obtained by hydrodistillation, were characterized by GC-FID and GC/MS analyses. The highest number of components (40) was identified in *L. integrifolia* EO, which, along with that of *A. seriphioides*, contained important amounts of oxygenated monoterpenes (44.35 and 29.72%, resp.). Thymol (27.61%) and carvacrol (13.24%) were the main components of *A. seriphioides* EO, and borneol, lippifoli-1(6)-en-5-one, and terpinen-4-ol (>8.5%) were the principal compounds of *L. integrifolia* EO. These results support the idea that oxygenated monoterpenes are the bioactive fractions of the EOs. Finally, the study shows that these Andean species might be used to treat superficial fungal infections and to improve the local *Chagas* disease situation by vector-control.

Introduction. – Plants from Andean regions are genuine biological sources of a variety of natural products biosynthesized in response to the abiotic and biotic stress of surviving in such extreme conditions. Over the last years, there have been important changes in land use in the Central Andes, province of San Juan, Argentina. This mountain area is under intense human activity due to the extraction of metal minerals such as gold by open-sky mining. In turn, there has been a great loss of natural vegetation. Therefore, phytochemical analyses of Andean plant species must be carried out urgently to make a survey of the biodiversity of this region, its composition, and its utility, before it is definitely lost.

The province of San Juan is located on the Andean slopes in central and western Argentina (latitude 31° S and longitude 69° W). The mountains along the province border are over 3000 m.a.s.l, and the valleys range from low snowy areas to desert areas with scarce rainfall. Thus, the native flora comprises a large number of species distributed throughout different ecosystems [1]. The province has a rich folk medicine tradition using an ample variety of medicinal plants [2]. These species are used as food condiments, infusions, or decoctions to treat liver problems, stomach disorders, ulcers, skin infections, and domestic pests. They belong to the Asteraceae, Apiaceae, Verbenaceae, and Lamiaceae families, phytochemically recognized by their important production of essential oils (EOs). EOs and their components have many functional properties in mammals and other organisms (insects, fungi, bacteria, and viruses) [3]. They are complex mixtures of components that sometimes present higher activities than the isolated compounds, in which case their final activity is due to the combined effects of several major or minor components. The natural product industry is actively seeking for natural therapeutics, preservatives, repellents, and other agents to replace synthetic compounds [4].

In the Central Andes of San Juan, plant species have to face extreme climatic conditions like excessive variations in temperature, desiccation, ultraviolet degradation, H_2O stress, and exposure to herbivores. Thus, pronounced changes in the composition of their EOs and their biological activities are expected, compared to species common in other ecoregions of Argentina. In this study, the biological activities such as antifungal, antibacterial, and repellent properties of six medicinal plant species from the Central Andes area were reported, and the chemical composition of three of them was described.

Results and Discussion. – *Chemical Composition of the Essential Oils.* The EOs were obtained by hydrodistillation of the air-dried parts. *Table 1* shows the yields [% (*w*/*w*)] and the physical properties of the EOs for the different species investigated. The highest EO contents were found in *S. parvifolia* (2.5%), followed by *L. integrifolia* (1.5%), and *G. polycephalum* (1.15%).

Table 1. *Botanical and Vernacular Names of the Studied Medicinal Plants from the Central Andes of Argentina, and Yields and Physical Properties of Their Essential Oils*

Botanical name (family)	Vernacular name	Yield [% (*v*/*w*)]	δ^{25} [mg/ml]	n_D^{25}	$[\alpha]_D^{25}$
Acantholippia seriphioides (Verbenaceae)	Tomillo	0.75	0.864	1.4835	+0.71 (c = 0.420, $CHCl_3$)
Artemisia mendozana (Asteraceae)	Ajenjo	0.62	0.670	1.4840	–
Gymnophyton polycephalum (Apiaceae)	Bio-bio	1.15	0.870	1.4770	+41.82 (c = 0.416, MeOH)
Lippia integrifolia (Verbenaceae)	Incayuyo	1.50	0.950	1.4850	+12.05 (c = 0.448, $CHCl_3$)
Satureja parvifolia (Labiatae)	Muña muña	2.40	0.990	1.4780	–
Tagetes mendocina (Asteraceae)	Quinchihue	0.81	0.940	1.4990	−2.70 (c = 0.074, $CHCl_3$)

Tables 2–4 show the components and relative percentages obtained by GC (*RI*) and GC/MS analyses of the EOs of *A. seriphioides*, *G. polycephalum*, and *L. integrifolia*, respectively. In total, 22, 16, and 40 compounds were identified in the EOs of these species, respectively. The dominant components in the monoterpene-hydrocarbon fraction (51.64%) of *A. seriphioides* EO were *p*-cymene (21.57%) and γ-terpinene (14.99%). There was also a high content of oxygenated monoterpenes (44.35%), with thymol (27.61%) and carvacrol (13.24%) as the main components (*Table 2*). The chemical composition of this EO was similar, although slightly different, to those observed in other studies. For example, *Gillij et al.* [5] reported *p*-cymene (52.8%) and thymol (46.8%) as the main compounds for *A. seriphiodes* EO from the central region of Argentina, and *Ruffinengo et al.* [6] reported thymol (29.2%) as the main component in plants collected in northern Patagonia, Argentina. *Fuselli et al.* [7], reported thymol (29.2%), *p*-cymene (23.3%), and carvacrol (23.3%) as the main components in specimens collected in San Luis, another province in the arid region of Cuyo.

Table 2. *Chemical Composition of* Acantholippia seriphioides *Essential Oil*

Compound	*RI*[a])	Composition [%][b])	Identification[c])
α-Thujene	931	0.03	Ms_1
α-Pinene	940	0.84	Ms_1, Co
α-Fenchene[d])	950	0.55	Ms_1
Camphene[d])	952	0.18	Ms_1
Sabinene	972	0.09	Ms_1
Myrcene	992	4.08	Ms_1, Co
α-Phellandrene	1005	0.49	Ms_1
α-Terpinene	1018	5.58	Ms_1
p-Cymene	1025	21.57	Ms_1, Co
δ-3-Carene[d])	1029	0.29	Ms_1
Limonene[d])	1029	2.04	Ms_1, Co
β-*trans*-Ocimene	1051	0.38	Ms_1
γ-Terpinene	1061	14.99	Ms_1, Co
Terpinolene	1088	0.53	Ms_1
Borneol	1170	0.25	Ms_1
Terpinen-4-ol	1177	2.68	Ms_1, Co
Thymol methyl ester	1235	0.57	Ms_1, Co
Thymol	1290	27.61	Ms_1, Co
Carvacrol	1300	13.24	Ms_1, Co
β-Caryophyllene	1419	1.44	Ms_1
β-*cis*-Farnesene	1443	0.29	Ms_1
Caryophyllene oxide	1585	0.25	Ms_1
Total		*97.97*	

[a]) *RI:* Experimental retention indices determined on the *DB-5* column relative to homologous *n*-alkanes. [b]) Percentages were calculated from the peak area without correction. [c]) Mode of identification: Ms_1, Mass spectra and *RI* identical with those of pure reference compounds; Co, co-injection with authentic compounds. [d]) Critical co-elutions on the *DB-5* column were resolved by adding the second dimension polarity separation on the *Supelcowax 10* column.

Table 3. *Chemical Composition of* Gymnophyton polycephalum *Essential Oil*

Compound	*RI*[a])	Composition [%][b])	Identification[c])
α-Thujene	931	0.82	Ms_2
α-Pinene	940	1.63	Ms_1, Co
Camphene	950	31.84	Ms_1, Co
β-Myrcene	992	2.06	Ms_1, Co
α-Phellandrene	1005	10.2	Ms_1, Co
α-Terpinene	1018	0.5	Ms_2
p-Cymene	1025	3.55	Ms_1, Co
Limonene	1029	9.40	Ms_1, Co
β-*cis*-Ocimene	1038	12.60	Ms_2
β-*trans*-Ocimene	1051	15.34	Ms_1, Co
γ-Terpinene	1061	5.5	Ms_2
Terpinolene	1088	0.87	Ms_2
Terpinen-4-ol	1177	1.11	Ms_1, Co
β-Caryophyllene	1418	0.65	Ms_2
α-Muurolene	1499	0.45	Ms_2
Spathulenol	1578	0.22	Ms_2
Total		*96.74*	

[a]) *RI:* Experimental retention indices determined on the *DB-5* column relative to homologous *n*-alkanes. [b]) Percentages were calculated from the peak area without correction. [c]) Mode of identification: Ms_1, Mass spectra and *RI* identical with those of pure reference compounds; Ms_2, mass spectra and *RI* identical with literature data [8]; Co, co-injection with authentic compounds.

Table 3 shows the EO composition for *G. polycephalum* Gillies & Hook., which was characterized by high amounts of camphene (31.84%), α-phellandrene (10.2%), β-*cis*-ocimene (12.60%), and β-*trans*-ocimene (15.34%). Hydrocarbon monoterpenes comprised 94.31% of the oil of this species.

The EO from *L. integrifolia* was rich in both, hydrocarbon monoterpenes (30.25%) and oxygenated monoterpenes (29.72%), while hydrocarbon and oxygenated sesquiterpenes were present in similar amounts (16.79 and 16.63%, resp.; *Table 4*). The following compounds were present in concentrations between 4.51 and 8.72%: α-pinene, camphene, limonene, borneol, terpinen-4-ol, Δ^8-africanene, α-terpinyl acetate, lippifoli-1(6)-en-5-one, and caryophyllene oxide. The composition reported for *L. integrifolia* EO from a population of the central region of Argentina showed camphor (16.20%), Δ^8-africanene (5.04%), lippifoli-1(6)-en-5-one (16.70%), and α-farnesene (14.89%) as the main constituents [9].

The EO composition for the other species studied, *i.e.*, *A. mendozana*, *S. parvifolia*, and *T. mendocina*, has been reported in previous studies by our institute [10–12]. In total, 36 components were identified in *A. mendozana* EO representing 92.8% of the oil. The main compounds were camphor (22.4%), artemiseole (11.1%), artemisia alcohol (10.8%), and borneol (7.2%) [10]. This composition was in agreement with that reported by *Shatar et al.* [13], who reported the content and chemical composition of the EOs of eleven *Artemisia* species and found that camphor was the main component in six of them.

Table 4. *Chemical Composition of* Lippia integrifolia *Essential Oil*

Compound	*RI*[a])	Composition [%][b])	Identification[c])
α-Thujene	931	0.24	Ms_2
α-Pinene	940	7.91	Ms_1, Co
Camphene	950	6.61	Ms_1, Co
Sabinene	972	0.59	Ms_2
β-Pinene	980	0.3	Ms_2
Myrcene	992	0.83	Ms_2
α-Phellandrene	1005	0.12	Ms_2
α-Terpinene	1018	1.79	Ms_2
p-Cymene	1025	2.28	Ms_1, Co
Limonene[d])	1029	5.73	Ms_1, Co
1,8-Cineole[d])	1030	0.11	Ms_1, Co
γ-Terpinene	1061	3.02	Ms_2
cis-Sabinene hydrate	1070	1.96	Ms_1, Co
Terpinolene	1088	0.83	Ms_2
trans-Sabinene hydrate	1098	1.09	Ms_1, Co
Camphor	1146	2.87	Ms_1, Co
Borneol	1170	8.72	Ms_1, Co
Terpinen-4-ol	1177	8.33	Ms_1, Co
α-Terpineol	1188	0.48	Ms_2
cis-Carveol	1230	0.75	Ms_2
Bornyl acetate	1290	0.23	Ms_2
δ-Elemene	1338	0.68	Ms_2
$\Delta^{9(15)}$-Africanene	1345	1.07	Ms_1, Co
Δ^{8}-Africanene	1348	6.11	Ms_1, Co
α-Terpinyl acetate	1350	5.18	Ms_2
β-Bourbonene	1389	0.1	Ms_2
Longifolene	1408	1.21	Ms_2
β-Caryophyllene	1419	2.13	Ms_2
β-Gurjunene	1434	0.26	Ms_2
Aromadendrene	1441	0.28	Ms_2
α-Humulene	1455	0.99	Ms_2
Germacrene D	1487	1.35	Ms_2
Bicyclogermacrene	1500	0.64	Ms_2
β-Himachalene	1505	1.97	Ms_2
Lippifoli-1(6)-en-5-one (Africanone)	1553	8.71	Ms_1, Co
Spathulenol	1580	0.22	Ms_2
Caryophyllene oxide	1585	4.51	Ms_2
τ-Cadinol	1640	0.99	Ms_2
α-Cadinol	1652	0.43	Ms_2
Integrifoliane-1,5-dione (integrifolone)	1658	1.77	Ms_1, Co
Total		*93.39*	

[a]) *RI:* Experimental retention indices determined on the *DB-5* column relative to homologous *n*-alkanes. [b]) Percentages were calculated from the peak area without correction. [c]) Mode of identification: Ms_1, Mass spectra and *RI* identical with those of pure reference compounds; Ms_2, mass spectra and *RI* identical with literature data [8]; Co, co-injection with authentic compounds. [d]) Critical co-elutions on the *DB-5* column were resolved by adding the second dimension polarity separation on the *Supelcowax 10* column.

The EO of *S. parvifolia* revealed an important fraction of oxygenated monoterpenes (88.5%) from a total of 26 constituents that represented 94.1% of the oil. The major components were piperitone (34.9%), piperitenone (27.3%), *cis*-piperitenone epoxide (15.0%), and piperitenone oxide (6.0%) [11]. *Zygadlo et al.* [14] reported piperitenone oxide (69.8%), piperitenone (5.6%), and pulegone (4.4%) as the main constituents of *S. parvifolia* EO collected from Central Argentina. *Viturro et al.* [15] reported differences in the EO composition of *Satureja* species from Northeast Argentina (province of Jujuy), which was probably due to different chemotypes or to the influence of the soil, the altitude, and the weather conditions.

T. mendocina EO contained 21 constituents (97.3% of the total composition), with the major compounds (concentrations $>3.5\%$) being β-*trans*-ocimene, *cis*-tagetone, *trans*-tagetone, *cis*-ocimenone, α-pinene, and *trans*-ocimenone [12], which are the main recognized constituents of the EOs in marigold (*Tagetes*) species [16–18].

EOs could be a potential source of valuable monoterpenes for the industry [3][4][19]. To our knowledge, this is the first report on the chemical composition of EOs from *A. seriphioides*, *G. polycephalum*, and *L. integrifolia* collected in the Central Andes piedmonts of the San Juan Province (Argentina).

Antifungal Activity of the Essential Oils. Over the past two decades, fungal infections have been a major cause of morbidity and often mortality in immunocompromised patients [20]. The limited efficacy and high toxicity of the available antifungal drugs [21] have evidenced the need for new, alternative antifungal compounds. *Table 5* shows the *in vitro* antifungal activity of *A. seriphioides*, *A. mendozana*, *G. polycephalum*, *S. parvifolia*, and *L. integrifolia* EOs against several yeasts and filamentous fungi, determined with the broth microdilution method. All the tested EOs showed antifungal activity and, interestingly, they all showed fungicide rather than fungistatic properties.

Regarding the sensitivity of the fungal strains, dermatophytes were the most susceptible species. *S. parvifolia* EO showed the best activity against *Microsporum gypseum* (*MIC* = 31.2 μg/ml), followed by *Trichophyton mentagrophytes* and *T. rubrum* (*MIC* = 125 μg/ml). *G. polycephalum* EO inhibited *T. rubrum* and *T. mentagrophytes* with *MIC* values of 250 μg/ml. *G. polycephalum*, *L. integrifolia*, *A. seriphioides*, and *S. parvifolia* EOs showed the best *MIC* values against *T. rubrum* and *T. mentagrophytes*, which are responsible for more than 93% of the superficial fungal infections worldwide and very difficult to eradicate. The most active EOs against the first panel of fungal strains were tested against a second panel containing six clinical isolates of each species of *T. mentagrophytes* and *T. rubrum* (*Table 6*). The aim was to gain an insight in the antifungal spectrum of these EOs not only on standardized, but also on clinical strains isolated from patients of health care centers in Argentina, and to assess the possibility of developing these EOs for clinical antifungal use. The results show (*Table 6*) that *A. seriphioides*, *G. polycephalum*, *L. integrifolia*, and *S. parvifolia* EOs showed fungistatic and fungicide activities against almost all the clinical strains (*MIC* and *MFC* values between 62.5 and 1000 μg/ml). The activity against this panel of clinical isolates was similar to that observed against the standardized strains. *A. seriphioides* EO showed a *MIC* value eight-times lower against the clinical isolate *T. mentagrophytes* (C364) than against the standardized *T. mentagrophytes* strain ATCC 9972 (*Tables 5* and *6*). Moreover, this EO showed a fungicide activity against *T. mentagrophytes* C364 at a 16-times lower concentration than against the strain ATCC 9972.

Table 5. *Antifungal Activity of the Essential Oils of Medicinal Plants from the Central Andes of Argentina in Comparison to the Reference Compounds Thymol, Amphotericine B, Ketoconazole, and Terbinafine*

Microorganism	*A. seriphioides*		*A. mendozana*		*G. polycephalum*		*S. parvifolia*		*L. integrifolia*		Thymol	Amphotericine B	Ketoconazole	Terbinafine
	MIC[a])	*MFC*[a])	*MIC*	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*	*MIC*	*MIC*	*MIC*	*MIC*
C. albicans	125	250	500	750	500	500	–[b])	–	1000	1000	125	1.0	0.5	n.d.[c])
C. tropicalis	125	250	500	750	500	1000	–	–	1000	1000	125	0.5	0.125	n.d.
S. cerevisiae	250	500	–	–	62.5	62.5	–	–	–	–	125	0.5	0.5	n.d.
C. neoformans	125	250	–	–	500	–	1000	–	1000	–	62.5	0.25	0.25	n.d.
A. flavus	1000	1000	–	–	500	500	–	–	–	–	n.d.	0.5	0.125	n.d.
A. niger	1000	1000	–	–	–	–	–	–	–	–	n.d.	0.5	0.5	n.d.
A. fumigatus	1000	1000	–	–	1000	1000	1000	1000	–	–	n.d.	0.5	0.25	n.d.
M. gypseum	500	500	–	–	500	750	31.2	100	500	500	62.5	0.125	0.04	0.04
T. rubrum	500	1000	–	–	250	500	125	250	500	1000	62.5	0.075	0.025	0.01
T. mentagrophytes	500	1000	–	–	250	250	125	250	500	1000	62.5	0.075	0.025	0.04

[a]) The antifungal activity was assessed with the broth microdilution methods M27-A2 and M38-A recommended by the NCCLS [22][23] and expressed as minimal inhibitory concentration (*MIC*) and minimal fungicidal concentration (*MFC*) in [μg/ml]. [b]) –: Not active (*MIC* or *MFC* > 1000 μg/ml). [c]) n.d.: Not determined.

Table 6. *Antifungal Activity of the Essential Oils of Medicinal Plants from the Central Andes of Argentina against Clinical Dermatophyte Isolates in Comparison to the Reference Compound Terbinafine*

Clinical Dermatophyte Isolates	*A. seriphioides*		*G. polycephalum*		*L. integrifolia*		*S. parvifolia*		Terbinafine
	MIC[a])	*MFC*[a])	*MIC*	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*	*MIC*
T. rubrum C 110	1000	1000	500	500	250	250	250	500	0.06
T. rubrum C 135	250	250	125	250	1000	1000	125	125	0.06
T. rubrum C 136	250	250	250	250	125	250	250	250	0.06
T. rubrum C 137	500	1000	125	125	250	250	125	500	0.06
T. rubrum C 139	1000	1000	500	500	250	250	500	500	0.12
T. rubrum C 140	1000	>1000	500	1000	1000	1000	250	250	0.03
T. mentagrophytes C 108	1000	>1000	500	1000	500	500	250	1000	0.06
T. mentagrophytes C 364	62.5	62.5	250	250	500	500	500	500	0.06
T. mentagrophytes C 539	125	250	500	500	250	250	125	250	0.06
T. mentagrophytes C 738	500	500	500	1000	1000	1000	500	1000	0.06
T. mentagrophytes C 943	500	500	250	250	250	1000	125	250	0.06
T. mentagrophytes C 726	500	1000	250	250	500	1000	250	500	0.12

[a]) The antifungal activity was assessed with the broth microdilution method M38-A recommended by the NCCLS [23] and expressed as minimal inhibitory concentration (*MIC*) and minimal fungicidal concentration (*MFC*) in [μg/ml].

It is worthwhile to take into account that *A. seriphioides* and *G. polycephalum* EOs were active against the yeasts *Candida albicans*, *C. tropicalis*, *Saccharomyces cerevisiae*, and *Cryptococcus neoformans* (*MIC* and *MFC* values between 62.5 and 1000 μg/ml). The best activity against *S. cerevisiae* (*MIC* and *MFC* = 62.5 μg/ml) was exhibited by *G. polycephalum* EO, which was, together with the EO of *A. seriphioides*, the only active EO against the filamentous fungi *Aspergillus flavus* and *A. fumigatus* with *MIC* and *MFC* values between 500 to 1000 μg/ml. The percentage of thymol and carvacrol in *A. seriphioides* EO is high (27.61 and 13.24%, resp.). Both compounds have been widely recognized to have fungicide and bactericide activities in previous works, including studies of the mechanisms of action [24][25]. Based on the present results, the EOs of *A. seriphioides*, *G. polycephalum*, *S. parvifolia*, and *L. integrifolia* collected in the Central Andes area of Argentina are clearly promising sources of compounds for treating superficial fungal infections.

Antibacterial Activity of the Essential Oils. The antibacterial activity of the EOs isolated from *A. seriphioides*, *G. polycephalum*, and *L. integrifolia* against a panel of *Gram*-positive and *Gram*-negative bacteria was assessed with the broth microdilution method, and the EOs showed *MIC*s between 500 and 1000 μg/ml (*Table 7*). *A. seriphioides* EO was the most effective among the three EOs studied. It showed inhibitory activity against both *Gram*-positive *S. aureus* strains and all the *Gram*-negative bacteria tested, with *MIC* values between 500 and 750 μg/ml. The clinical

isolate *E. coli* LM_2 was the most sensitive bacterium ($MIC = 500$ μg/ml) to *L. integrifolia* EO. EOs cross the cell walls and the cytoplasmic membranes by disrupting the structure of the different layers of polysaccharides, fatty acids, and phospholipids, which in turn permeabilizes them [3]. The *MIC* values of thymol against the tested bacteria are shown in *Table 7*. The antibacterial activity of *A. seriphioides* EO could be due to the important content of thymol (27.61%).

Table 7. *Antibacterial Activity of the Essential Oils of Medicinal Plants from the Central Andes of Argentina in Comparison to the Reference Compounds Cefotaxime and Thymol*

Bacteria	Minimal inhibitory concentration [μg/ml]				
	A. seriphioides	*G. polycephalum*	*L. integrifolia*	Cefotaxime	Thymol
Staphylococcus aureus methicillin-sensitive ATCC 29213	750	–[a])	1000	0.5	250
Staphylococcus aureus methicillin-resistant ATCC 43300	750	750	1000	0.5	250
Escherichia coli ATCC 25922	500	750	1000	0.5	250
Escherichia coli LM_1	500	750	1000	5.0	125
Escherichia coli LM_2	750	750	500	0.5	125
Pseudomonas aeruginosa ATCC 27853	750	750	1000	7.5	500
Yersinia enterocolitica PI	750	–	1000	0.5	125
Salmonella enteritidis MI	750	750	1000	12.5	250
Salmonella sp LM	750	750	1000	0.5	250

[a]) –: Not active (> 1000 μg/ml).

Repellent Activity of the Essential Oils. The repellent activity against *Triatoma infestans* (triatomine bugs), a *Chagas* disease vector, of the EOs isolated from six species of the Central Andes of Argentina, *i.e.*, *A. seriphioides*, *A. mendozana*, *G. polycephalum*, *L. integrifolia*, *S. parvifolia*, and *T. mendocina*, is summarized in *Table 8*. Significant differences in the average repellent activity were observed among the EOs, and the majority of them showed excellent repellent properties against the triatomines (*Table 8*).

Two of the six EOs were found to be *Class V* repellents, as the positive control tetrametrine, and three others were *Class IV* repellents, the categories of maximum and high repellency, respectively. Based on the repellence percentage (RP) in a 72-h treatment, the most repellent EOs were those of *L. integrifolia* (60 to 100% RP) and *G. polycephalum* (68 to 100% RP). *A. seriphioides* EO was a weak repellent with attractant properties at 72 h. Triatomine bugs are vectors of the protozoan parasite *Trypanosoma cruzi* that causes *Chagas* disease, which is widely distributed in Central and South America with 7.7 million persons currently infected. According to the *World Health Organization*, the disease causes 12,500 deaths per year. One of the main control strategies for eliminating *Chagas* disease is based on controlling transmission by domestic and peridomestic vectors. The number of new cases a year due to vectorial

Table 8. *Repellent Activity* [%] *of the Essential Oils of Medicinal Plants from the Central Andes of Argentina against Nymphs of* Triatoma infestans

Essential oil	Repellency [%][a])			Average repellency [%]	Class[b])
	1 h	24 h	72 h		
Control[c])	−12±34.4	−28±32	−100±0.0	−46.7±17.8 (a)[d])	–
A. seriphioides	60±12.6	12±34.4	−20±49.0	17.33±20.8 (ab)	*I*
A. mendozana	100±0.0	60±17.9	60±17.9	73.33±9.3 (bc)	*IV*
G. polycephalum	68±15.0	92±8.0	100±0	86.7±6.4 (c)	*V*
L. integrifolia	60±0.0	100±0.0	100±0.0	86.6±5.0 (c)	*V*
S. parvifolia	100±0.0	76±16.0	12±26.5	62.6±13.8 (bc)	*IV*
T. mendocina	84±16.0	76±24.0	76±24.0	78.7±11.6 (c)	*IV*
Tetrametrine[e])	100±0.0	100±0.0	100±0.0	100.0±0.0	*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 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 ($p < 0.05$). [e]) Positive control at a concentration of 0.2% (*w*/*v*).

transmission is 41,200 (7.775 per 100,000) [26]. The prevalence of *T. infestans* in specific areas of northern Argentina, Bolivia, and Paraguay are recurrent after spraying with pyrethroid insecticides. Additionally, resistance has been reported during the last decade [27]. At present, the use of synthetic chemicals for controlling insects and arthropods raises several environmental and human health concerns. The use of environmentally friendly natural products with good efficacy is an extremely attractive alternative. EOs appear to be a valuable natural source of repellent agents [19]. Some EOs from Argentinian species with repellent properties were recently reported. *Toloza et al.* [28][29] evaluated the repellent activity of EOs from native and exotic Argentinian plants and isolated three alcohols (benzyl alcohol, menthol, and thymol) that were effective against *Pediculus humanus capitis*. Likewise, insecticidal properties for a number of EOs from Bolivian plants have been reported by *Laurent et al.* [30], among them several species from the *Lippia* and *Satureja* genera. One study evaluated the repellent and toxic properties of *Schinus molle* extracts (Anacardiaceae) from Argentina, but did not mention its chemical composition [31].

Conclusions. – The EOs of *A. seriphioides*, *G. polycephalum* and *S. parvifolia* collected in the Central Andes region of Argentina are promising sources of compounds for treating dermatophyte-related infections. Moreover, the strong repellent activity of *L. integrifolia* and *G. polycephalum* EOs showed that these Andean species could be used as a new potential source for fighting the local problem of *Chagas* disease by vector control. To our knowledge, this is the first report on the chemical composition of EOs from *A. seriphioides*, *G. polycephalum*, and *L. integrifolia* collected in the Central Andes of the Province of San Juan, Argentina. The Asteraceae (*Artemisia* and *Tagetes* sp.), Labiatae (*Satureja* sp.), and Verbenacae (*Lippia* and *Accantholippia* sp.) families are widely recognized as botanical families of aromatic and

medicinal herbs. The chemical variability of the composition of their members is highly dependent on edaphic and climatic conditions. The anthropic pressure related to big-scale exploitation of minerals is threatening the survival of wild species in the Andean mountain range of San Juan. Some of the species reported here are common to other arid and semiarid regions of the Earth, but the chemical variability of the EOs could influence the specific biological properties we observed in this study.

The authors are grateful to the *ANPCyT* (PICTs 995, 2007-01468, 2010-0608), the *UNR* and the *CICITCA UNSJ* for their financial support. *B. L.*, *L. L.*, and *M. B. A.* hold fellowships from the *CONICET*. *S. L.* and *M. B. A.* are doctoral students in *PROBIOL-UNCuyo*.

Experimental Part

Plant Material. Acantholippia seriphioides (A. Gray) Mold (Verbenaceae), *Artemisia mendozana* DC (Asteraceae), *Gymnophyton polycephalum* (Gillies & Hook.) Clos. (Apiaceae), *Satureja parvifolia* Phil. Epling (Lamiaceae), *Tagetes mendocina* Phil. (Asteraceae), and *Lippia integrifolia* Griseb. Hieron. (Verbenaceae) were collected from the Iglesia district in the Central Andes area, province of San Juan, Argentina, during the 2007–2008 flowering period. The species were identified by Dr. *Luis Ariza Espinar* (*Instituto Multidisciplinario de Biología Vegetal*, *CONICET*, and Universidad Nacional de Córdoba), and voucher specimens were deposited with the *Museo Botánico de Córdoba*, Argentina.

Isolation of Essential Oils. The aerial parts of the species (500 g each) were hydrodistilled in a *Clevenger*-type apparatus for 1 h, according to the method recommended by the *European Pharmacopoeia* [32]. The EOs were dried (anh. Na_2SO_4) and stored at $-18°$ in the dark prior to analysis. The yields and the physical properties (δ^{25}, n_D^{25}, and $[\alpha]_D^{25}$) of the EOs were also determined.

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 × 0.25 mm i.d., film thickness 0.25 μm) coated with a non polar 5% phenyl/95% dimethylpolysiloxane phase. The oven temp. was programmed from 40 to 230° at 2°/min; injector and detector temp., 240°; carrier gas, N_2 (0.9 ml/min). The identification of the components was based on the comparison of their retention indices (*RI*s) with those of a homologous series of *n*-alkanes (C_9–C_{25}) and of pure authentic samples.

GC/MS Analysis. The GC/MS analyses were carried out with a *Perkin Elmer Q-700* apparatus equipped with an apolar *DB-5* fused-silica cap. column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temp. was programmed from 40 to 230° at 2°/min; carrier gas, He (0.9 ml/min); ion source, 70 eV. The oil components were identified by using two computer MS libraries (*Wiley* and *Nist*) with *RI*s as a preselection routine, by visual inspection and comparison of the mass spectra with those of the *Adams* spectral library for confirmation [8], and by co-injection with authentic compounds.

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 110, *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) [22][23], 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 [22][23] 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 $\leq 1\%$. Ketoconazole, terbinafine, amphotericin B, and thymol were used as positive controls. Minimum fungicidal concentrations (*MFC*s), defined as the lowest concentrations of EOs that produce total inhibition of visible growth, was determined as follows. After determining the *MIC*, an aliquot of 5 μl of sample was withdrawn from each clear well of the microtiter plate and transferred onto a 150-mm *RPMI-1640* agar plate buffered with *MOPS* (*Remel*, Lenexa, Kansas). The inoculated plates were incubated at 30° and *MFC*s were recorded after 48 h.

Antibacterial Activity. The *MIC* values were determined using the microbroth dilution method according to the protocols of the NCCLS [33]. 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 (*Argentina 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.

Repellent Activity against Triatoma infestans. The bioassays were carried out according to *Talukder* and *Howse* [34]. Halved filter-paper discs (9 cm in diameter) 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. A circular halved filter-paper disc, with one half treated with 0.5 ml of acetone and the other one untreated, was used as a control. After solvent evaporation, the filter-paper discs were placed in *Petri* dishes. Five starved fifth instar *T. infestans* nymphs were released in the centre of each *Petri* dish and maintained under controlled conditions of temp. (22°), humidity (60%), and a 16 h light/8 h darkness photoperiod. Experiments were performed in quintuplicate. The insects and the repellence control *Espacial 0.2*® (tetrametrine 0.2% (*w*/*w*)) were provided by the *Servicio Nacional de Chagas* (Córdoba, Argentina). The insect distribution was recorded after 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 data were analyzed with the *Kruskal Wallis* test. Comparisons and mean rank separations were performed according to *Conover et al.* [35] at $p = 0.05$, using the statistical software Infostat/E (version 2.0). The mean values of RP 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%) [34].

REFERENCES

[1] G. E. Feresin, A. Tapia, A. Gutierrez Ravelo, C. Delporte, N. Backhouse Erazo, G. Schmeda-Hirschmann, *J. Pharm. Pharmacol*. **2002**, *54*, 835.
[2] D. Bustos, A. Tapia, G. E. Feresin, L. Ariza Espinar, *Fitoterapia* **1996**, *67*, 411.
[3] F. Bakkali, S. Averbeck, D. Averbeck, M. Idaomar, *Food Chem. Toxicol*. **2008**, *46*, 446.
[4] A. R. Koroch, R. H. Juliani, J. A. Zygadlo, in 'Flavours and Fragances', Springer, Berlin, Heidelberg, 2007, p. 87.

[5] Y. G. Gillij, R. M. Gleiser, J. A. Zygadlo, *Bioresour. Technol.* **2008**, *99*, 2507.
[6] S. A. Ruffinengo, M. B. Eguaras, I. C. Floris, C. D. Faverin, P. E. Bailac, M. E. Ponzi, *J. Econ. Entomol*. **2005**, *98*, 651.
[7] S. R. Fuselli, S. B. García de la Rosa, M. J. Eguaras, R. Fritz, M. Mndagijimana, L. Vannini, M. E. Guerzoni, *J. Essent. Oil Res.* **2007**, *19*, 514.
[8] R. P. Adams, 'Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy', 3rd edn., Allured Publishing Corp., Carol Stream, Illinois, 2001.
[9] A. Velasco-Negueruela, M. J. Pérez-Alonso, C. A. Guzmán, J. A. Zygadlo, *J. Essent. Oil. Res.* **1993**, *5*, 513.
[10] B. Lima, M. P. de Lampasona, C. Schuff, A. Tapia, R. Bomben, C. Duschatzky, G. E. Feresin, *J. Essent. Oil Bear. Plants* **2008**, *11*, 496.
[11] L. Luna, B. Lima, A. Tapia, G. E. Feresin, C. Duschatzky, M. Possetto, M. P. de Lampasona, C. Schuff, *J. Essent. Oil Bear. Plants* **2008**, *11*, 106.
[12] B. Lima, M. B. Agüero, J. A. Zygadlo, A. Tapia, C. Solis, A. Rojas de Arias, G. Yaluff, S. Zacchino, G. E. Feresin, G. Schmeda-Hirschmann, *J. Chil. Chem. Soc*. **2009**, *54*, 68.
[13] S. Shatar, S. Altantsetseg, S. Darijimaa, *J. Essent. Oil Bear. Plants* **2006**, *9*, 22.
[14] J. A. Zygadlo, F. Merino, M. Maestri, C. Guzmán, *J. Essent. Oil. Res.* **1993**, *5*, 549.
[15] C. I. Viturro, A. C. Molina, I. Guy, B. Charles, H. Guinaudeau, A. Fournet, *Flavour Fragrance J.* **2000**, *15*, 377.
[16] A. Gil, C. M. Ghersa, S. Leicach, *Biochem. Syst. Ecol.* **2000**, *28*, 261.
[17] J. A. Zygadlo, N. R. Grosso, R. E. Abburra, C. A. Guzman, *Biochem. Syst. Ecol.* **1990**, *18*, 405.
[18] M. L. López, N. E. Bonzani, J. A. Zygadlo, *Biochem. Syst. Ecol.* **2008**, *36*, 882.
[19] L. S. Nerio, J. Olivero-Verbel, E. Stashenko, *Bioresour. Technol.* **2010**, *101*, 372.
[20] D. P. Kontoyiannis, E. Mantadakis, G. Samonis, *J. Hosp. Infect.* **2003**, *53*, 243.
[21] T. F. Patterson, *Lancet* **2005**, *366*, 1013.
[22] NCCLS (National Committee for Clinical and Laboratory Standards), 'Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved standard M 27-A2', 2nd edn., Wayne, PA, 2002.
[23] NCCLS (National Committee for Clinical and Laboratory Standards), 'Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi, Approved standard M 38-A', 2nd edn., Wayne, PA, 2002.
[24] A. Ben Arfa, S. Combes, L. Preziosi-Belloy, N. Gontard, P. Chalier, *Lett. Appl. Microbiol.* **2006**, *43*, 149.
[25] S. Bennis, F. Chami, N. Chami, T. Bouchikhi, A. Remmal, *Lett. Appl. Microbiol.* **2004**, *38*, 454.
[26] World Health Organization, 'Special Program for Research and Training in Tropical Diseases: Reporte Sobre la Enfermedad de Chagas', Buenos Aires, Argentina, 2007.
[27] S. L. Vallvé, H. Rojo, C. Wisnivesky-Colli, *Mem. Inst. Oswaldo Cruz* **1996**, *91*, 405.
[28] A. C. Toloza, J. Zygadlo, G. Mougabure Cueto, F. Biurrun, E. Zerba, M. I. Picollo, *J. Med. Entomol.* **2006**, *43*, 889.
[29] A. C. Toloza, A. Lucia, E. Zerba, H. Masuh, M. I. Picollo, *Bioresour. Technol.* **2008**, *99*, 7341.
[30] D. Laurent, L. A. Vilaseca, J.-M. Chantraine, C. Ballivan, G. Saavedra, R. Ibañez, *Phytother. Res.* **1997**, *11*, 285.
[31] A. Ferrero, J. O. Werdin González, C. Sánchez Chopa, *Fitoterapia* **2006**, *77*, 381.
[32] Council of Europe (COE) – European Directorate for the Quality of Medicines, in 'European Pharmacopeia', 5th edn., Strasbourg, 2005, Vol. 1, p. 217.
[33] NCCLS (National Committee for Clinical and Laboratory Standards), 'Performance Standards for Antimicrobial Susceptibility Testing, 8th Informational Supplement, Document M100-S18', Wayne, PA, 2008.
[34] F. A. Talukder, P. E. Howse, *Int. J. Pest Manage.* **1994**, *40*, 274.
[35] W. J. Conover, 'Practical Nonparametric Statistics', John Wiley & Sons, Inc., New York, 1999.

Received August 5, 2010