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CHARACTERIZATION OF THE ESSENTIAL OIL AND EXTRACTS FROM THE AERIAL PARTS OF KEHUIÑA (*POLYLEPIS BESSERI*)

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Abstract. The genus *Polylepis* (Rosaceae: Sanguisorbeae) includes about 20 species of shrubs and small to medium-sized trees. In Bolivia, Kessler recognized nine species and eight subspecies. *Polylepis* forests represent a wood resource in a region where no other trees grow, playing important ecological roles such as the protection of soil against erosion. The bark of *Polylepis*, commonly known as kehuiña, kewiña, queñua, keuña or q'iwiña, is used in folk medicine against coughs, rheumatism or arthritic pain. The resin is also reported as being effective against urinary diseases. In this work, the essential oil of *Polylepis besseri* Hieron ssp. besseri was analyzed by GC and GC/MS. The essential oil was then fractionated by centrifugal chromatography in order to confirm (by GC/MS) the identification of the diterpene fraction. The study showed that the main components of the essential oil were the diterpenes abietadiene (40,3%) and abietatriene (6,1%). The antibacterial activity of the essential oil and extracts obtained from dried and pulverized leaves of *Polylepis besseri besseri* was evaluated using *Shigella flexneri, Escherichia coli, Staphylococcus aureus*, and *Bacillus subtilis*. Its insecticidal activity was also evaluated on Aedes aegypti larvae and *Triatoma infestans* nymphs. *Accepted 24 August 2002.*

Key words: Polylepis besseri besseri, *Rosaceae, essential oil composition, abietadiene, abietatriene, antibacterial activity, insecticidal activity.*

INTRODUCTION

The genus *Polylepis* (Rosaceae: Sanguisorbeae) includes about 20 species of shrubs and small to medium-sized trees (Jordan 1980 in Kessler 1995). In Bolivia, Kessler recognized nine species and eight subspecies (Kessler 1995). *Polylepis* forests represent a wood resource in a region where no other trees grow, playing important ecological roles such as the protection of soil against erosion. The bark of *Polylepis*, commonly known as kehuiña, kewiña, queñua, keuña or q'iwiña (Girault 1984, Killeen *et al.* 1993), has also been used in folk medicine against coughs, rheumatism, gout, and arthritic pain (De Lucca & Zalles 1992, Sagaseta & Uranga de Ilurdoz 1996), as has the resin which is reported to be effective against urinary diseases (De Lucca & Zalles 1992).

While some *Polylepis* spp. have been widely studied as medicinal plants (Catalano *et al.* 1994, Catalano *et al.* 1995), to our knowledge their essential oil composition has not been studied so far.

Polylepis b. besseri is a tree around 8 m tall, characterized by a dense layer of white woolly hairs on the flowering stands, outside of the flowers, and on the fruits. Hybrids of *P. besseri*, with *P. tomentella* ssp. *tomentella* and *P. tomentella* ssp. *incanoides* are known and greatly complicate identification. *P. besseri* flowers throughout the year, but some populations may have no flowering individuals at any time of the year (Fjeldså & Kessler 1996).

Polylepis b. besseri is distributed at 3000–4100 m in Cochabamba and Chuquisaca, Bolivia, where *P. tomentella*, *P. incanoides*, *P. racemosa* ssp. *lanata*, and *P. neglecta* can also be found (Fjeldså & Kessler, 1996).

Polylepis forests have some very important ecological function. Forests on mountain ridges with persistent wind-driven fog "comb" moisture out of the atmosphere. These forests may also act like a sponge, storing large amounts of water in the vegetation and organic soils during the summer and releasing it gradually during the dry season. They also moderate runoff and protect the soil against erosion (Fjeldså & Kessler 1996).

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Antimicrobial and antifungal activities of essential oils have been described by many authors (Pellecuer *et al.* 1976, Mallea *et al.* 1979, Yousef & Tawil 1980, Deans 1991, Beuchat 1994). Many other studies have examined the effects of essential oils on bacteria for a wide range of applications (Deans 1991). The purpose of this study was to examine the inhibitory effect of *Polylepis* essential oil and extracts against selected microorganisms in order to verify its use in folk medicine.

Furthermore, parasitic diseases such as malaria, yellow fever and chagas represent a serious health problem in most developing countries. Chagas disease is transmitted to humans by some Triatominae species which may differ from one region to another, *Triatoma infestans* being the most widespread vector. The eradication of the *Triatominae* in the infected regions being therefore represents one of the methods of preventing the disease.

Synthethic pyrethroids are among the most effective insecticides against Triatominae, but the cost of the vector control is beyond the financial resources of some countries. In Bolivia, many local plants are used by rural people against plant pests and insects (Laurent *et al.* 1997). All these plants have an aromatic character and contain essential oils that could be responsible for the observed activities. In the present work the insecticidal activity of *Polylepis* essential oil and extracts was studied on *Aedes aegypti* and *Triatoma infestans*.

METHODS

Material collection and essential oil isolation. Samples of fresh aerial parts (leaves and stems) of *Polylepis b. besseri* were collected in "Alto Sapanani" at an altitude of 3600 m. during the spring season of 1997. The samples taken were representative of the species and its geographic area of distribution.

Voucher specimens of the plant were identified and deposited at the Herbarium of the National Flora Reserve "Martín Cárdenas" in Cochabamba (Torrico BOLV 346). A pale yellow oil, with a d^{20} 0.8420, was obtained from the fresh aerial parts by steam distillation over 12–16 h at atmospheric pressure (564 mm Hg) using a Clevenger-type glass hydrodistillation apparatus (Guenther 1972). The yield ranged from 0.08 to 0.11% w/w on dry basis.

The extracts were obtained following the procedure shown in Fig. 1.

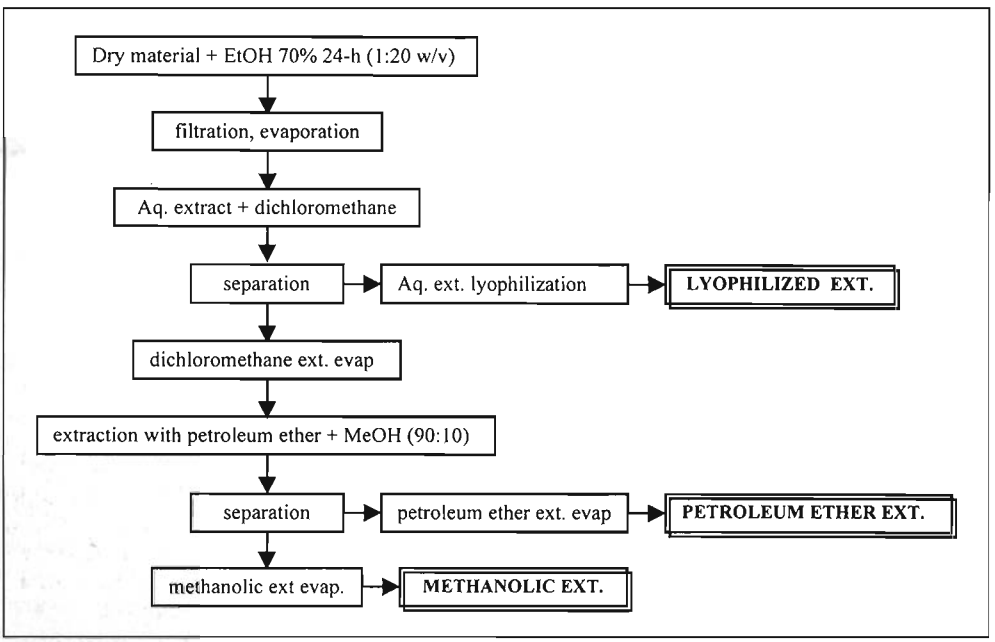

FIG. 1. Extracts preparation for biological activity test.

Chromatographic analyses. GC analysis. The composition of the essential oil was determined by GC using a Shimadzu 14 B gas chromatograph equipped with an FID and a Shimadzu data processor software EZ-Chrom, using an SE-52 (Mega, Legnano, Italy) cross-linked fused-silica capillary column (25 m x 0.32 mm), coated with 5% phenyl-polymethylsiloxane (0.40–0.45 µm phase thickness), under the following conditions: 60°C for 8 min, then 60°–180°C at 3°C/min, 180°–250°C at 20°C/min and finally 250°C for 10 min. injector temperature 250°C; detector temperature 280°C; injection mode, split; split ratio 1:30; volume injected 0.2 µl of the sample. Carrier gas was hydrogen at 55 kPa.

The analysis was also carried out using a Carbowax 20M (Ohio Valley, USA) bonded fused-silica capillary column (25 m x 0.32 mm), coated with polyethylene glycol (0.25 µm phase thickness), under the following conditions: 40°C for 8 min, then 60°–180°C at 3°C/min, finally 180°–230°C at 20°C/min. injector temperature 250°C; detector temperature 250°C; injection mode split; split ratio 1:30; volume injected 0.2 µl of the sample. Carrier gas was hydrogen at 30 kPa.

GC/MS analysis. GC/MS analysis was performed on a Shimadzu QP 5050 gas chromatograph equipped with reference libraries (McLafferty & Stauffer 1991, Adams 1995) using two capillary columns. The first was an SE-52 (Mega, Legnano, Italy) cross-linked fused-silica capillary column (25 m x 0.25 mm), coated with 5% phenyl-polymethylsiloxane (0.25 µm phase thickness), under the following conditions: 60°C for 8 min, then 60°–180°C at 3°C/min and finally 180°–230°C at 20°C/min. injector temperature 250°C; detector temperature 280°C; injection mode split; split ratio 1:40; volume injected 0.2 µl of the sample. Helium was used as a carrier at 122.2 kPa; interface temperature 250°C; acquisition mass range 40–400 m/z. The second capillary column was a BP 20 (SGE, Australia) bonded fused-silica (25 m x 0.25 mm), coated with polyethylene glycol (0.25 µm phase thickness), under the following conditions: 40°C for 8 min, then 40°–180°C at 3°C/min and finally 180°–230°C at 20°C/min. injector temperature 250°C; injection mode split; split ratio 1:40; volume injected 0.2 µl of the sample. Carrier gas was helium at 92.6 kPa; interface temperature 250°C; acquisition mass range 40–400 m/z.

Biological tests. Microorganisms. Inhibition assays were carried out following Mitscher's method (Mitscher *et al.*1971) with cultures of *Shigella flexneri*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. All cultures were grown in Trypticase Soy Agar (TSA) and Trytic Soy Broth (TSB) sterilized at 121°C (15 min), cultivated at the Instituto de Investigaciones Farmaco-Bioquímicas, where the antibacterial activities were assessed.

Insects. Aedes aegypti larvae (Bora Bora strain) were reared in the laboratory under controlled temperature (28° ± 1°C), and fed with sterilized powdered cat food. *Triatoma infestans* were also reared in the laboratory under controlled temperature (28° ± 1°C) and relative humidity (80% HR ± 10%).

Test on *Aedes aegypti* larvae. Solutions of the essential oil were prepared in ethanol at concentrations of 20 and 50 g/l. Twenty late 3rd or young 4th-instar mosquito larvae were collected from the rearing basin and transferred into plastic glasses containing 99 ml of filtered tap water. The essential oil was tested at final concentrations of 10 and 50 mg/l. The volume of ethanol added to each glass was adjusted to 1 ml. Each concentration was tested using four glasses with 20 larvae. A control was treated in the same way with no sample. Mortality was recorded after 24 hours and corrected if necessary using the Abbot formula (Abbot 1925). These tests were carried out in duplicate or triplicate.

Topical application on *Triatoma infestans* nymphs. The *Polylepis* extracts and essential oil were used as solutions in ethanol with concentrations of 2% and 20% (v/v). 1 µl of each solution was applied directly over the abdomen of ten 4th-instar nymphs, 24 h later mortality was monitored and the nymphs were re-treated 72 h later with 5 µl of the same solution. Two sets of controls were utilized. One control group was treated with ethanol only, while the other was not treated at all. The effect of the application (knock-down or mortality) was observed for another week and compared with the controls. Insects were considered to be knocked-down if they lay on their backs and were unable to right themselves when disturbed. This experiment was carried out in duplicate on different days.

RESULTS AND DISCUSSION

The chromatogram of *Polylepis b. besseri* essential oil obtained on an SE-52 column is shown in Fig. 2. Its composition as single components is reported in

TABLE 1. Percentage composition of the essential oil of *Polylepis b. besseri*.

	compound*	% **	SE-52***	CW***
1	α-pinene	0.1	926	1024
2	sabinene	0.1	971	1100
3	β-pinene	0.1	971	1044
4	octanal	0.1	1002	
5	*p*-cymene	1.8	1022	1219
6	limonene	0.7	1026	1158
7	1,8-cineole	2.4	1029	1179
8	(*Z*)- β-ocimene	0.3	1044	1200
9	(E)- β-ocimene	0.2	1049	1200
10	γ-terpinene	0.3	1055	
11	fenchone	0.4	1084	1346
12	linalool	0.4	1100	1524
13	nonanal	0.8	1103	1413
14	menthone	2.3	1153	1442
15	isomenthone	0.6	1161	1413
16	menthol	0.9	1175	
17	terpinen-4-ol	0.5	1175	1558
18	*p*-cymen-8-ol	0.1	1184	
19	α-terpineol	0.3	1191	1662
20	myrtenol	0.2	1200	
21	pulegone	0.2	1233	1549
22	geraniol	0.1	1255	
23	undecanal	0.2	1306	
24	α-copaene	0.2	1372	1435
25	β-bourbonene	0.1	1380	
26	dodecanal	0.2	1405	
27	β-caryophyllene	0.2	1411	1573
28	9-epi-β-caryophyllene	0.2	1451	
29	γ-muurolene	0.3	1468	
30	α-muurolene	1.0	1491	1660
31	δ-cadinene	2.4	1512	1685
32	α-cadinene	0.2	1529	1771
33	(*E*)-nerolidol	0.5	1558	1987
34	spathulenol	1.2	1569	2065
35	1-epi-cubenol	0.4	1621	
36	epi-α-muurolol	3.8	1639	2120
37	pimaradiene	4.1	1950	
38	α-cadinol	3.8	1651	2167
39	M.W. 272[a]	8.8	1972	
40	sclarene	1.0	1975	
41	abietatriene	6.1	2044	2429
42	abietadiene	40.3	2130	2395
43	abietol	2.6	2395	

* The components are reported according their elution order on SE-52

** These percentages were obtained on SE-52 except for sabinene, β-pinene, limonene and 1,8-cineole which were obtained on Carbowax 20M

*** L.R.I. = Linear Retention Indices

a) MS, m/z (%): 272 [M]+ (27), 257 (100), 133 (29), 105 (33), 91 (56)(36), 55 (44), 43 (70), 41 (97)

Table 1. Forty-three components, which represent 90% of the total composition, were identified. Individual components were identified by comparison of their Linear Retention Indices on two columns with those from pure standards or reported in the literature. Comparison of fragmentation patterns in the mass spectra with those stored on the spectrometer database (McLafferty & Stauffer 1991, Adams 1995) was also performed. The percentages of each component were expressed as raw percentages without standardization. The experimental results in Table 1 showed that the oil featured a high diterpene content (54%), and among the oxygenated compounds α-cadinol (3.8%) and abietol (2.6%). To our knowledge, the low presence of monoterpenes (11.7%) and sesquiterpenes (14.1%) (oxygenated and linear hydrocarbons) in the essential oil is unusual (Loayza *et al.* 1999, Lorenzo *et al.* 2001, Lorenzo *et al.* 2002).

The antibacterial activity of the essential oil and extracts of *Polylepis b. besseri* was evaluated in the laboratories of the Instituto de Investigaciones Farmaco-Bioquímicas, using *Shigella flexneri*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. The results are shown in Table 2, indicating low antimicrobial activity depending on the different samples evaluated.

The results reported in Table 3 show the insecticidal effects of *Polylepis* extracts and essential oil on *Aedes aegypti* larvae and *Triatoma infestans* 4th-instar nymphs. The insecticidal activity of many essential oils is known to be mainly due to the presence of mono- and sesquiterpenes such as linalool, nerolidol, anethol, limonene, etc. (Chantraine *et al.* 1998). However, the essential oil of *Polylepis b. besseri* is shown to be poor in these components and its insecticidal activity could perhaps be attributed to the presence of the diterpenes abietadiene and abietatriene (Palevitch & Craker 1994). This hypothesis could be confirmed by assessing the insecticidal activity of these diterpenes.

The toxicity of sesquiterpenes and diterpenes as insecticides against mosquitoes (Palevitch & Craker 1994), and as repellents for yellow fever mosquitoes (Hwang *et al.* 1985) is already known.

In accordance with the above results, other species of the genus *Polylepis* accompanying *P. besseri* in its habitat will be delaminated in order to test their composition. Moreover, a more detailed investigation of the different species of *Polylepis* should be performed to evaluate the presence of such rich diterpene fractions, to elucidate by other spectroscopic methods

TABLE 2. Antibacterial activity of the essential oil and extracts of *Polylepis b. besseri.*

Sample	*Shigella flexneri*	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus subtilis*
Essential oil	NE	NE	NE	NE
Petroleum ether extract	–	–	+	+
Methanolic extract	–	–	+	+
Lyophilized extract	+	–	–	–

+ = active – = inactive NE = not evaluated

structures not identified by their GC/MS patterns, and to define taxonomic implications, both botanically and chemically.

TABLE 3. Insecticidal activity of the essential oil and extracts of *Polylepis b. besseri.*

Sample	*Aedes aegypti*	*Triatoma infestans*
Essential oil	++	+
Petroleum ether extract	++	–
Methanolic extract	+++	–
Lyophilized extract	–	–

+ = mortality > 80% at 100 mg/L ++ = mortality >80% at 50 mg/L +++ = mortality > 80% at 10 mg/L – = inactive

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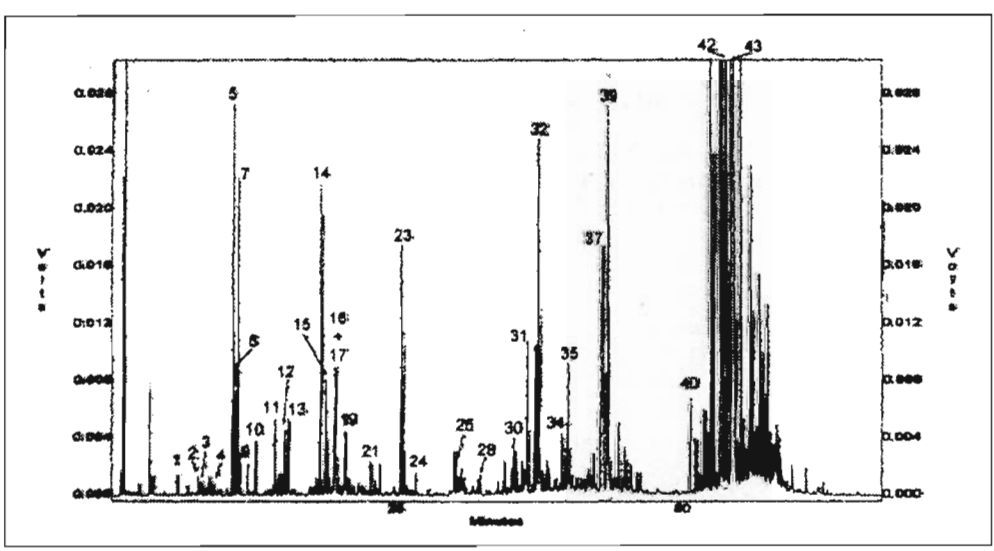

FIG. 2. Chromatogram of *Polylepis b. besseri* essential oil on SE 52.

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