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Essential oils from Bolivia. xiv. *Lamiaceae*: *Clinopodium axillare*

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Abstract
The chemical composition of the essential oil obtained from two subspecies of *Clinopodium axillare* growing wild in the High Valley Region of Bolivia, Province of Cochabamba, was determined by a combination of GC and GC/MS measurements. It is dominated by oxygenated monoterpenes. Piperitenone oxide (20-30 %), piperitone epoxide (15-19 %), piperitenone (13 %), pulegone (3-5 %) and piperitone (4-5 %) as well as limonene (8-12 %) and □-pinene (1-5 %) are the major constituents. These results, in conjunction with a recent description of the genus *Clinopodium*, provide a better understanding of the classification of this plant.

Keywords: *Clinopodium axillare, Satureja sp., Lamiaceae, Essential oil composition, piperitenone oxide, piperitenone, piperitone epoxide*

1. Introduction
In 1998, with the help of IDRC and the involvement of a research team of the Universidad Mayor de San Simón, Cochabamba, Bolivia, an extensive survey was launched on the aromatic plants of the High Valley region of this country. South America, has a very rich, diversified, and somehow unknown flora. A series of several papers were produced to highlight a small part of its potential: the essential oils [1]. Due to the lack of information, one of these plants was identified as *Satureja* sp., or occasionally called *S. boliviana*. The results of the oil composition stayed on our shelves. A major review of the genus *Clinopodium* in Bolivia has recently been published [2]. This paper has provided a better understanding of the taxonomy and systematics on this small genus: our two samples were identified one as *Clinopodium axillare* subsp. *axillare* (Rusby) Harley and the second *C. axillare* subsp. *uniflorum* (Rusby ex Briq) JRI Wood.
Organic solvents and aqueous extracts of *S. boliviana* and *S.* sp. are active against HSV-1 and VSV viruses [3-4]. Both plants were collected in different regions of Bolivia and possibly are not the same as discussed here. Medicinal uses for digestive problems of Burro muña (see below) are described in literature [5].

2. Experimental
2.1 Plant Material
Clinopodium axillare (syn. *Satureja axillaris*) and its subsp. are described in ref. (2). They are aromatic shrubs. *C. axillare* subsp. *axillare*, (local name: Burro muña), was collected in Quiriría, San José, Province of Esteban Arce, (altitude: 2796 m, 17°54'54"S and 65°56'06"W, 10 Feb. 1999) (sample a in Table1).
A second sample B in Table 1, *C. axillare* subsp. *uniflorum*, was collected in Arani, near Rodeo adentro, Province of Carrasco, 3050 m, 17 Feb. 1998 (sample B in Table 1). Voucher specimens have been deposited in the Herbarium Nacional Forestal Martin Cardenas (BOLV) of the Universidad Mayor de San Simón, Cochabamba: codes Ramirez 61 and Arrazola 194, respectively.

2.2 Oil Extraction
In the A case (Table 1), 55 kg of the aerial parts of the plant material were introduced into the distillation pot for 1.5 hours. It gave 108 mL of oil, producing a yield of 0.2 % in (volume /weight). Sample B of the plant was submitted to similar procedure.

2.3 GC and GC/MS

Essential oils were analyzed in duplicate by GC on a gas chromatograph HP 5890 with an automatic injector HP 7683 and FID detector equipped with two columns: a polar Supelcowax 10 and a non-polar DB-5 fused silica capillary column (30 m □ 0.25 mm □ 0.25 μm). Detector and injection temperatures were 220 and 260°C, respectively. The oils were also analyzed by GC–MS on a HP 5972 mass spectrometer at 70 eV coupled to a HP 5890 GC equipped with the same columns as above. The temperature program for both GC/FID and GC/MS was from 40 °C (2 min) to 210 °C (33 min). The carrier gas was He at a flow of 1.4 mL/min; inlet pressure: 104.5 kPa. Injection volume and split ratio: 3 μL and (50:1). Operating conditions for the MS: the carrier gas was He at a flow of 1.0 mL/min; inlet pressure 48.5 kPa; temperature of the source 280 °C and scan speed, 0.6 s between 40 and 450 amu. Identification of the components was done by comparison of their retention indices (RI) with normal hydrocarbons with even number of C atoms ranging from C8 to C30 and by comparison of their mass spectra with literature data [6-8] and with our own data bases. Quantitative data were obtained electronically from GC/FID area percentages.

Table 1: Composition of the essential oil of the aerial part of *Clinopodium axillare* from Bolivia

Column		**DB-5**	**S-wax 10**		
	Identification	R. I.	R. I.	A	B
1	Ethyl isovalerate	843	1053	t**	t
	-Thujene	912	1012	t	t
	-Pinene	918	1008	1.0	4.7
4	Camphene	933	1037	0.1	0.2
5	Thuja-2,4(10)-diene	941	1107	-	t
6	Sabinene	963	1087	0.4	0.5
	-Pinene	966	1072	1.0	2.1
8	3-Octanone	982	1242	-	0.1
9	Myrcene	988	1144	0.7	0.7
10	-Phellandrene	1000*	1134	-	t
11	3-Octanol	1000*	1382	-	0.3
12	*p*-Mentha-1(7),8-diene	1000*	1142	t	t
	^{3}-Carene	1006	1142	-	0.3
14	-Terpinene	1012	1153	-	t
15	*p*-Cymene	1020	1252	t	t
16	Limonene	1026	1175	12.4	9.2
17	1,8-Cinéole	1027	1189	t	0.1
	-Phellandrene	1026	1183	-	0.1
19	*cis*--Ocimene	1036	1224	0.1	0.2
20	*trans*-Ocimene	1046	1238	0.5	0.9
21	-Terpinene	1054	1232	t	0.1
22	Phenethyl methyl ether	1078	1464	0.0_5	0.0_5
23	Terpinolene	1084	1264	0.0_5	t
24	Linalool	1098	1532	0.9	0.3
25 ol	*trans*-Mentha-2,8-dien-1-	1116	1625		0.0_5
26	3-Octanol acetate	1125	1323	-	0.2
27	Menthone	1147	1437	1.8	2.8
28	Isomenthone	1157	1458	0.9	1.3
29	Isopulegone *	1172	1555	0.1	0.1
30	Terpinen-4-ol *	1172	1577	-	t
31	-Terpineol	1186	1675	0.4	0.4
32	8,9-Dihydrothymol	1209	1975	0.2	0.1
33	Pulegone	1235	1619	3.5	5.3
34	Piperitone	1247	1693	5.1	4.2
35	*cis*-Piperitone epoxide	1248	1680	6.2	4.4
36	*trans*-Piperitone epoxide	1250	1699	13.2	10.3
37	2-Phenylethyl acetate	1255	1784	0.7	0.1
38	Isopiperitenone	1266	1803	0.4	0.4
39	Unidentified (i), see text	1272	1818	0.6	0.3
40	Bornyl acetate	1283	1550	0.1	0.2
41	Thymol	1296	2156	t	t
42	Myrtenyl acetate	1304	1657	0.2	0.1
43	*trans*-Carvyl acetate	1334	1709	0.2	0.4
44	Piperitenone	1341	1888	12.9	12.7
45	*cis*-Carvyl acetate	1361	1713	-	0.0_5
46	Piperitenone oxide	1373	1923	29.2	19.8
48	(*E*)-Jasmone	1387	1899	t	0.1
48	-Caryophyllene	1412	1562	3.0	3.2
49	2,5-Dimethoxy-*p*-cymene	1423	1844	-	0.1
	-Humulene	1449	1633	0.1	0.2
51	Alloaromadendrene	1455	1608	0.1	t
52	Unidentified (ii)	1456	1985	0.4	0.4

53	Isomer of #52	1460	2000	t	0.1
54	Germacrene D	1476	1675	-	0.0_5
55	Bicyclo germacrene	1491	1697	1.8	1.3
56	(*E*)-Guaiene	1497	1691	-	0.1
57	-Bisabolene	1507	1700	-	0.4
58	-Cadinene	1510	1727	t	0.9
59	*trans*-Calamenene	1520	1797	-	0.1
60	-Cadinene	1520	1727	0.1	0.2
61	-Sesquiphellandrene	1523	1742	-	0.1
62	-Cadinene	1534	1759	-	t
63	(*E*)-Bisabolene	1543	1748	-	t
64	(*E*)-Nerolidol	1563	2017	-	0.1
65	Germacrene D-4-ol	1572*	2015	0.2	0.2
66	Spathulenol	1572*	2081	-	0.1
67	Caryophyllene oxide	1574	1932	0.1	0.3
68	Viridiflorol	1585	2042	-	0.3
69	Humulene epoxide II	1601	1984	-	t
70	1,10-Di-epi-cubenol	1609	2017	-	0.9
71	-Cadinol	1636	2143	0.1	3.2
72	-Eudesmol	1652	2174	-	0.2
73	-Cadinol	1652	2228	-	0.0_5
74	Bulnesol	1670	2168	-	0.0_5
75	*epi*- or -Bisabolol	1682	2187	-	2.8
	Total (%)			98.8	98.5

3. Results and discussion

Physical properties

The density of sample A is 0.884, its refractive index is 1.4935. The optical rotation of sample B is + 32.8º.

Chemical composition

The compositions appear in Table 1. Both samples are characterized by a high percentage of oxygenated monoterpenes. Piperitenone oxide (20-30%), piperitenone (13%), *trans*-piperitone epoxide (10-13%), *cis*-piperitone epoxide (4.5-6%), pulegone (3.5-5%), piperitone (4-5%), menthone (2-3%) and isomenthone (~ 1%) as well as limonene (8-12%) and -pinene (1-5%) are the major constituents. -Cadinol and - or *epi*--bisabolol (0-3% each) are the main sesquiterpenes. Two quantitatively important unknown compounds also are observed (Table1). The first one (RI (DB-5 column) = 1272) has a mass spectrum similar to that of piperitenone oxide and is proposed to be isopiperitenone oxide (Fig. 1). In support of this proposal, the retention indices for piperitenone and piperitenone oxide, RI (DB-5), on the DB-5 a-polar column are 1341 and 1366, respectively. This is a difference of + 25 units. The same difference is +7 between isopiperitenone and the first unidentified compound. Similar observation can be observed can be made on the polar Supelcowax column. To our knowledge, isopiperitenone oxide was observed only once in the *Mentha longifolia* oil in Jordan [9]. The reported analysis is made on the a-polar SPB-5 column. Unfortunately, there is no RI reported values and, more disturbing, although there is no indication on the order of elution, one may infer that the list of measured compounds follow the order of elution, which does not correspond to our list. The second unknown compound, RI (DB-5) = 1456, has a molecular weight of 170 amu. It could be an oxygenated monoterpene molecule such as $C_{10}H_{18}O_2$. This compound is followed by a smaller peak, ratio ~1/6, which has a very similar mass spectrum.

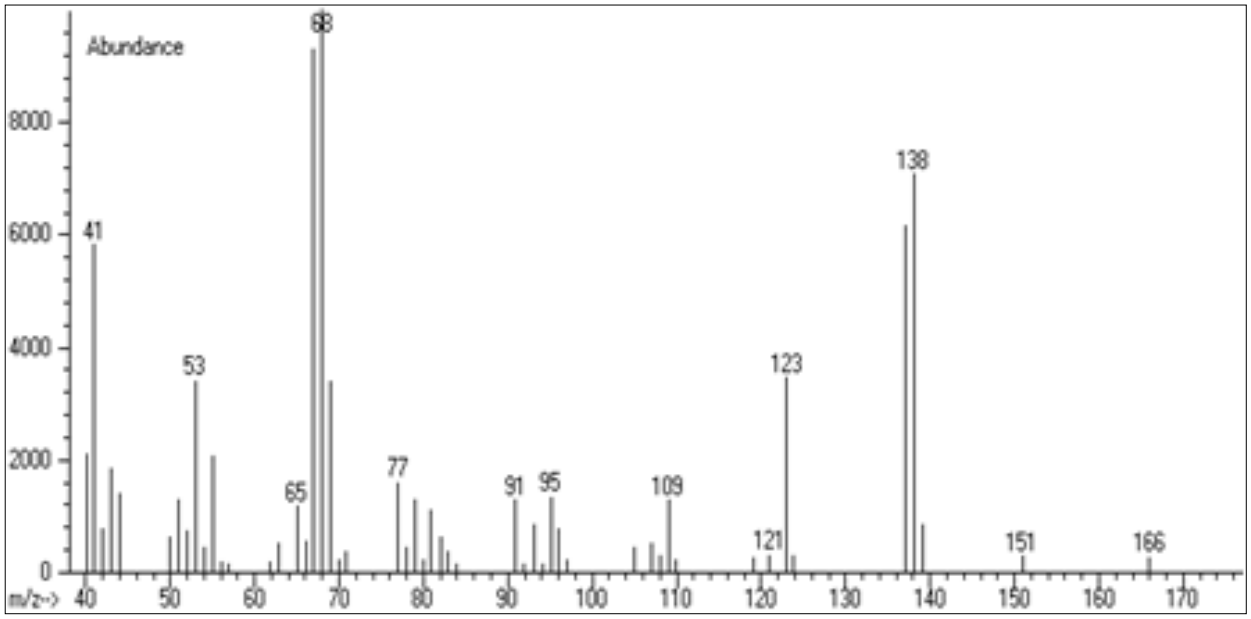

Fig 1: Mass spectrum of the unidentified compound, RI (DB-5) = 1272. See text.

Fig 2: Location of samples described in this paper. A and B are the samples studied here and numbers, 10 to 17, refer to the referenced papers.

It should be noted that the main compounds present in the oil of *S. boliviana* Briq. Obtained from northern Argentina, in the Jujuy region (about 500 km South of Cochabamba) are □-terpinene (15.4%), □-caryophyllene (10.2%), germacrene-D (8.9%), and bicyclogermacrene (8.3%) (Fig. 2) [10]. Another *S. boliviana* sample collected in the Famatina Department, Argentina, shows the presence of isomenthone (33%) and pulegone (25.3%) [11]. On the North West side of Bolivia, in the Cusco Department, Peru, except for the presence of 2.1% of piperitone, there is no other piperitone derivatives [12]. The same observations were reported in two other papers [13-14S]. Thus, the samples studied here do not belong to the *S. boliviana* sp.

On the other hand, the oil obtained from *S. parvifolia* (Phil.) Epl. (syn. *S. gilliesii* (Graph.) Briq. And *Clinopodium gilliesii* (Benth.) Kuntze) in the same region of Jujuy, Argentina, shows piperitenone oxide (69.8%) and piperitone (5.6%) as the main compounds (Fig. 2) [10]. In the province of Córdoba, Argentina (about 1300 km south of Cochabamba) piperitone oxide (30%), menthol (20%) and piperitenone oxide (15%) are reported as the main products [15]. Again, in the North-East of Argentina, in the Iglesia district, piperitone (34.9%), piperitenone oxide (27.3%), and *cis*-piperitenone epoxide (15.0%) are the main compounds [16]. In another sample, the partial analysis of the essential oil of *C. gilliesii* collected in La Rioja province of Argentina there is a mixture of pulegone (19.2%), isopulegone (12.0%) and piperitenone oxide (14.7%) [16]. It appears that the samples studied here have a closer relationship with those of *C. gilliesii* than those of *S. boliviana* sp. In disagreement with this statement, it should be mentioned that, in Chile, far more to the south, on the Pacific Ocean coast, the fresh leaves of *S. gilliesii* produces an essential oil very rich in isopulegyl acetate (25%), isopulegone (13%) and isopulegol (11%) [17]. More recently, the composition of the essential oil of *C. gilliesii* collected in La Rioja Province, Argentina, shows the presence of pulegone (19.2%), isopulegone (12.0%), and piperitenone oxide (14.7%) [18]. One may be tempted to see a transition between the rich piperitone and piperitenone oxide species and the Chilean rich isopulegone and isopulegol species.

To return to the taxonomy and systematics of *Clinopodium* mentioned above [2], the classification carried out on the characters of the plants such as leaf shape, leaf dentation, inflorescence form and branching, calyx size… are more reliable than essential oil analysis. In this study, it was concluded that *S. parvifolia*, *S. gilliesii* and *Clinopodium gilliesii* are synonyms. The two samples described here belong to the *Clinopodium axillare* family (syn. *S. axillaris*), to the subsp. *uniflorum* for the sample A and for the sample B to the subsp. *axillare*. It can be concluded that *S. parvifolia* and *S. axillare* have an essential oil rich in piperitone, piperitenone and their oxidized derivatives.

Other extracts obtained from *Satureja* species containing piperitone, piperitenone, and their oxides, were observed in other parts of the world. A few years ago, a partial survey of the main compounds identified in the essential oil of the *Satureja* species was published [19]. Half of them contain at least two of the following major compounds: □-terpinene, *p*-cymene, thymol, and carvacrol. Only the oil of three of them, *S. kallarica*, *S. paradoxa*, and *S. parviflora*, contain piperitone and piperitenone oxide. It must be added that this is also the case of the essential oil of *S. masukensis* and *S pseudosimensis* growing in Tanzania [20], *S. paradoxa* in Ethiopia [21], and the supercritical CO_2 extract of *S. fructicosa* from Spain [22]. Finally, let us mention that menthone (35%) and piperitenone oxide (31%) followed by linalool (5%) are the main compounds observed in the essential oil of the leaves of *Clinopodium macrostemum* var. *laevigatum* [23]. Thus, the *S. gilliesii* is not a unique rich piperitone and piperitenone oxides case among the *Clinopodium* family.

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