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Chemical constituents of essential oils of Muña, Bolivian plants traditionally used as pesticides, and their insecticidal properties against Chagas' disease vectors

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Abstract

The composition of essential oils from two muña, Bolivian medicinal plants, derived from *Minthostachys andina* and *Hedomea mandonianum*, were analyzed by gas chromatography/mass spectrometry. Major differences were observed in their chemical composition. Pulegone was the major component of *H. mandonianum* oil (44.6%) and *M. andina* oil (25.5%); menthone and isomenthone were around 33% of these oils. Differences were also observed in their insecticidal activity against the Chagas' disease vector, *Rhodnius neglectus* or *Triatoma infestans* bugs exposed on impregnated oil filter papers. While *M. andina* oil showed 30%–50% of mortality in both triatomine species after a period of 1 week, *H. mandonianum* oil did not show any insecticidal activity. Nevertheless, both species had insecticidal activity (33.3% and 50%) when oils were topically applied. The significance of these results is discussed in relation to the variability of the chemical composition and their potential use in Chasgas' disease vector control.

Keywords: muña; *Minthostachys andina*; *Hedomea mandonianum*; Labiatae; Essential oil composition; Insecticidal properties; Triatominae

1. Introduction

A number of plants of the Labiatae family growing in the Andean mountains at altitude between 2500 and 5000 m are called *muña* or *khoa* by the Kechuas Indians, *huaichcha* (Oblitas-Poblete, 1969) by the Aymara Indians and *poleo silvestre* by the Spanish people. This includes species of *Satureia*, *Minthostachys*, *Menta* and *Hedomea*. These plants, with a characteristic 'mint' scent, are used in traditional medicine in infusion (maté) to treat migraine, heart palpitations, rheumatisms, headache, height sickness (soroche), inflammation, anemia, fever, dysentery and also as an an

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tiseptic, insecticide and repellent (Bastien, 1983; Girault, 1984). The essential oils carefully prepared by the Indians are employed to protect the crops of potatoes from pests.

Chagas' disease is a particularly significant vector-borne disease problem in Bolivia where rural inhabitants are in contact with the parasite *Trypanosoma cruzi*. The main means of interrupting *T. cruzi* transmission is to control the vector, but national programmes have a lot of problems (Schofield and Dias, 1991) and reinfestation is very common in rural areas. The aim of this work was to investigate the constituents of two samples of essential oils of muña extracted from *Minthostachys andina* (Britton) Eppling and *Hedomea mandonianum* Wedd. and the insecticidal properties against the vectors of Chagas' disease, the triatominae bugs *Rhodnius neglectus* and *Triatoma infestans*.

2. Materials and methods

2.1. Plant material

Minsthostachys andina (Britton) Eppling and *Hedomea mandonianum* Wedd. were collected in Bolivia, near Chajaya, Department of La Paz at 3600 m; vouchers have been deposited in the National Herbarium of La Paz under acquisitions #AF 560 and #AF 599. The dry samples were steam-distilled for 3 h, the distillation water was saturated with hexane and the oil was collected in hexane.

2.2. Analytical techniques

Essential oils were diluted in methanol (1/20) and 0.5 ml was injected into a gas chromatography/mass spectrometry (GC/MS) set-up. GC/MS analysis was carried out on an Automass 120 mass spectrometer coupled with an ATI UNICAM 610 gas chromatograph. The GC was fitted with a polar column SGE BP20: 50 m × 0.22 mm, thickness 0.25 μm, injector 230°C; oven temperature programmed 60°C to 220°C at 3°C/min, constant temperature for 15 min; carrier gas helium, injector pression 14 lb/in^2 and with a non-polar column SGE BPX5 50 m × 0.22 mm, thickness 0.25 μm; temperature programmation was the same as above. The ion source temperature was 150°C while the ionisation potential was 70 eV (electron impact).

Retention indexes were obtained by injection of the homologous hydrocarbons series with the same temperature programmed run. Relative concentrations were calculated using peaks areas. Components were identified by comparison of their mass spectra and retention indexes with those already reported.

2.3. Insecticidal activity

2.3.1. Insects

Triatoma infestans or *Rhodnius neglectus* fourth-stage nymphs were bred in laboratory conditions using a technique previously described (Schmeda-Hirschmann and Rojas de Arias, 1992). They were maintained at 28°C and 60%–70% relative humidity. They were fed to repletion on living pigeons and after 6 h were exposed to the essential oils by topical application or exposed on treated filter papers. After 3 days, and thereafter weekly, nymphal mortality was recorded. The maximum observation time was fixed at 28 days, the period necessary for the moulting of all controls. In both topical application and filter paper exposure, insects were used in duplicate.

2.3.2. Pre-screening test

A topical test was used as a pre-screening in order to select the essential oils with insecticidal properties. Thereafter the selected essential oils were evaluated using the contact test.

2.3.2.1. Topical test. Essential oils have to be assayed at a high enough concentration to assess the effect of their minor constituents. Therefore, for screening purposes the oil dose to be applied on bugs was previously fixed at 50 μg of essential oil per insect (Schmeda-Hirschmann and Rojas de Arias, 1992). A stock of solution containing 50 mg of essential oil per ml of solvent was prepared for each sample and 1 μl of the solution was topically applied in duplicate on dorsal tergites of six insects. Bugs treated with solvent were used as controls.

Table 1
Chemical composition of *H. mandonianum* and *M. andina* oils

H. mandonianum oil				*M. andina* oil			
Time (min)	Molecular weight	Identification	%	Time (min)	Molecular weight	Identification	%
11.57	136	α-Thujene	0.41	11.57	136	α-Thujene	0.4
13.00	112	Me-3 cyclohexanone	0.34	12.58	112	Me-3 cyclohexanone	0.4
13.37	136	Sabinene	0.43	13.34	136	Sabinene	0.45
15.14	134	*p*-Cymene	0.6				
15.22	136	D-Limonene	0.41	15.22	136	D-Limonene	0.45
15.39	154	Eucalyptol	7.9	15.38	154	Eucalyptol	0.44
16.58	170	Linalyl oxide *cis*	0.39				
17.36	170	Linalyl oxide *trans*	0.36				
				17.51	136–154	Linalol	0.82
20.36	154	Menthone	2.62	20.41	154	Menthone	24.85
21.00	154	Iso-menthone	2.83	21.02	154	Iso-menthone	8.08
21.32	154	4-Terpineol	0.34				
22.05	138	α-Terpineol	0.47				
24.01	152	Pulegone	44.62	24.03	152	Pulegone	25.5
25.29	150	Thymol	0.5				
				26.21	168	Hexadecanol	1.07
27.29	196	Terpineol acetate	0.36				
27.51	150	Eucarvone	0.28	27.48	150	Eucarvone	0.92
29.08	204	β-Bourbonene	0.33				
36.52	220	Caryophyllene oxide	0.44	36.53	220	Caryophyllene oxide	1.71

2.3.2.2. Contact test. Thirty milligrams of essential oils was dissolved in 1.2 ml of acetone, applied to a filter paper (Whatman no. 1, 9 cm diameter) in the bottom of a Petri dish and allowed to dry. Ten insects were exposed in duplicate on treated filter papers. Bugs exposed on filter papers treated with 1 ml of acetone were used as controls.

2.3.2.3. Analysis of results. Mortality data calculated as averages were sorted by hand. Statistical analysis of the proportions was performed using the chi-square test for each exposure time and between two tests or the Fischer exact test when required.

3. Results and discussion

The chemical study was undertaken on two different columns by GC/MS. The different consti-

Table 2
Insecticidal activity of essential oils of muña against *Rhodnius neglectus* when topically applied ($n = 12$) and on filter paper ($n = 20$) exposure

Components	Test	Insect mortality (dead insects/total used)						
		24 h	48 h	72 h	7 days	14 days	21 days	28 days
Control	Topical	0/12	0/12	0/12	0/12	0/12	0/12	0/12
	Contact	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Essential oils of *M. andina*	Topical	0/12	0/12	4/12	6/12	6/12	6/12	6/12
	Contact	0/20	2/20	3/20	8/20	11/20	16/20	17/20*
Essential oils of *H. mandonianum*	Topical	0/12	0/12	4/12	4/12	4/12	4/12	4/12
	Contact	0/20	0/20	0/20	0/20	0/20	0/20	0/20

*Significantly different when the topical application was compared with the contact test: Fischer exact tests, $P < 0.05$.

Table 3
Insecticidal activity of *Mynthostachys andina* oil against *Triatoma infestans* ($n = 20$) applied on filter paper

	Insect mortality (dead insects/total used)						
Components	24 h	48 h	72 h	7 days	14 days	21 days	28 days
Control	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Essential oils of *Mynthostachys andina*	0/20	4/20	4/20	6/20	8/20	8/20	10/20
Essential oils of *Hedomea mandonianum*	0/20	0/20	0/20	0/20	0/20	0/20	0/20

tuents of the two samples were identified by comparison of mass spectra with library data (polar column) and by combination of mass spectra data and retention indexes for the non-polar column. The profiles of chromatograms are similar but the composition is different. The same components were identified in the two oils and are listed in Table 1. The amount of non-identified components was 27.5% for *Hedomea mandonianum* oil and 14% for *Minthosctachys andina* oil.

Nevertheless, the results exhibit great differences. Pulegone was detected as the major component of *Hedomea mandonianum* oil (44.6%) and the mixture of pulegone, isomenthone and menthone represents 50% of the essential oil. It is of interest to note that three compounds, menthone, isomenthone and pulegone, are present with around the same amount (58%) in *Minthostachys andina* oil, but in different proportions: 24.9%, 8.08% and 25.5%, respectively.

M. andina oil was the more active of the two essential oils tested. Active ingredients of both oils took around 1 week to be effective independently of the route of oil application (Table 2). When the applied tests were compared at different exposure times, significant differences were observed at 28 days of insect exposure. *H. mandonianum* oil did not present any insecticidal activity on filter paper in *R. neglectus* or *T. infestans* (Tables 2, 3). In this situation, it is unknown whether the insecticidal activity could be related to non-volatile compounds that remain on filter papers and kill triatomines that were exposed for a long period, or whether it was due to volatile compounds that could be still present in the saturated environment of the Petri dishes. It is important to note that *Rhodnius neglectus* showed a higher mortality percentage than *Triatoma infestans* when both were exposed on filter paper, demonstrating different sensitivities to these insecticidal compounds (Tables 2, 3).

Schmeda-Hirschmann and Rojas de Arias (1992) tested two samples of aeral parts of *Minthostachys* sp. (muña) and found no activity against *Rhodnius neglectus*. The great variability of chemical composition of muña essential oil could be explained by various factors; the variety of plants traditionally identified as muña (four genera), the altitude, the climate, the area of harvest, the season of harvest and the hour of harvest. Generally the essential oils are described as having antimicrobial and antiseptic properties (Janssen et al., 1987) and rarely for their insecticidal or repellent activities (Ahmad et al., 1993). However, there is unequivocal evidence that volatiles are detected by triatomines through their olfactory sensillar (Figueiras et al., 1994; Mayer, 1968; Taneja and Guerin, 1995). Therefore, volatile compounds of the essential oils with insecticidal properties could play an important role in triatomine control. It is not easy to recommend the use of muña essential oils for controlling vectors of Chagas' disease in rural households, because the chemical composition of the essential oils shows great variability. Further studies must evaluate the different compounds of the insecticidal species of muña in order to establish the standard chemical composition in insecticidal species, and to identify the insecticidal components by monitoring bioassays.

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