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Uses of hazardous medicinal plants: composition of the essential oil of *Clinopodium gilliesii* (Benth.) Kuntze (Lamiaceae), collected in Chile

[Uso de plantas medicinales peligrosas: composición del aceite esencial de *Clinopodium gilliesii* (Benth.) Kuntze (Lamiaceae), recolectada en Chile]

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Abstract: *Clinopodium gilliesii* (Benth.) Kuntze, harvested in the Chilean highlands, contains a surprising 93.87% of the toxic monoterpene pulegone in the essential oil. These results show remarkable differences with studies of the same species carried out in Argentina and Peru. These dissimilarities in the monoterpene composition of essential oils should be associated with differences in toxicity and biological activity of this medicinal plant used in ethnomedicine in different countries for the treatment of similar discomforts and diseases. These results are discussed considering the risk of consuming *C. gilliesii*, without clear recommendations and control of at least pulegone content in essential oils.

Keywords: *Clinopodium gilliesii*, essential oil, pulegone, toxicity

Resumen: *Clinopodium gilliesii* (Benth.) Kuntze, recolectada en el altiplano chileno, contiene un sorprendente 93,87% del monoterpeno toxico pulegona, en el aceite esencial. Estos resultados muestran diferencias notables con estudios de la misma especie realizados en Argentina y Perú. Estas disimilitudes, en la composición de los aceites esenciales deben estar asociadas con diferencias en la toxicidad y actividad biológica de esta especie medicinal que se utiliza en etnomedicina en diferentes lugares para el tratamiento de molestias y enfermedades similares. Estos resultados se discuten considerando el riesgo de consumir *C. gilliesii*, sin recomendaciones claras y control de al menos el contenido de pulegona en los aceites esenciales.

Palabras clave: *Clinopodium gilliesii*, aceite esencial, pulegona, toxicidad

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INTRODUCTION

Clinopodium gilliesii (Benth.) Kuntze, Lamiaceae, is a medium-sized aromatic shrub that can reach up to 1.5 m. It is commonly known as muña-muña and is geographically distributed in areas of the foothills of Peru, Bolivia, Chile and Argentina, between 2000 and 4500 m above sea level (Epling & Játiva, 1964). *C. gilliesii* presents a large number of synonyms, among them, *Micromeria gilliesii* Benth., *M. eugenioides* (Griseb.) Hieron., *Satureja gilliesii* (Benth.) Briq., and *S. parvifolia* (Phil.) Epling (Quattrocchi, 2012; Instituto de Botánica Darwinion, 2017; Kew Missouri Botanical Garden, The Plant List, 2017).

In Chile, it is distributed in the north, between the regions Arica-Parinacota and Atacama, although exceptionally its presence has also been mentioned in the center-south (Instituto de Botánica Darwinion, 2017).

In the Andean tradition, *C. gilliesii* has an important ethnobotanical value, emphasizing its use as condiment (Villagrán *et al.,* 2003) as well as its applications as a medicinal plant. The plant is traditionally used as infusion and according to folk medicine; muña-muña has been used to treat stomachache, coldindigestion, high-altitude illness, high blood pressure, heart pain, menopause and female infertility (Hieronymus, 1882; Kozel, 1991; Villagrán & Castro, 2003; Campos-Navarro & Scarpa, 2013; Ceballos & Perea, 2014). In Argentina, the plant has reputed aphrodisiac properties and it has been proved that dichlorometane extracts have relaxant effects on the smooth muscle of the *corpus cavernosum* of Guinea pigs. This property partially validates their aphrodisiac folk medicinal use (Hnatyszyn *et al.,* 2003).

The essential oil from *C. gillesii* has been studied by several authors and the results in composition have shown remarkable differences. Whereas piperitone, piperitenone, piperitenone oxide and *cis*-piperitenone epoxide (Viturro *et al.,* 2000; Luna *et al.,* 2008; Dambolena *et al.,* 2009) are characteristics components of some essential oils, carvacrol and carvacryl acetate are characteristics components of other essential oils (Muschietti *et al.,* 1996; Viturro *et al.,* 2007). Exceptionally and in < 20%, other monoterpenoids: (*E*)-Isocitral, pulegone, isopulegyl acetate, ρ-cymene, menthol, are also present (Zygadlo *et al.,* 1993; Muschietti *et al.,* 1996; Viturro *et al.,* 2007; Barbieri *et al.,* 2016). Although there are no studies demonstrating the cause of differences in the composition of essential oils, authors have suggested that possible chemical races and/or differences in abiotic factors from collection sites, such as soil composition, altitude and differences in climate, can be important factors influencing observed chemical dissimilarities. Differences in the chemical composition of essential oils are associated with potential toxicity risks and also with different healing properties of plants and plant extracts. This latter is a fact that so far has not been taken into account. Considering that this plant species is highly used in ethnomedicine in different countries, for treatments of discomforts and diseases, its potential toxicity is urgently required in order to improve its management for the treatment of human health.

In addition, and following our program in study essential oils of Northern Chile flora (Urzúa *et al.,* 2010; Urzúa *et al.,* 2011a; Urzúa *et al.,* 2011b; Urzúa *et al.,* 2013; Echeverría & Niemeyer, 2017), we describe for first time the analysis of the essential oil of *C. gilliesii*, collected in the Chilean highlands, which contains a surprising 93.87% of the toxic monoterpene pulegone. These results are discussed considering the risk of consuming *C. gilliesii*, without clear recommendations and control of at least pulegone content in essential oils.

EXPERIMENTAL

Plant Material

In March 2013, representative samples of leaves of *C. gilliesii* (Benth.) Kuntze were collected during the flowering season at Quebrada Luco, village of Murmuntani, (18° 21' 07" S, 69° 33' 05" W, and 4.000 m above sea level), Putre, Region of Arica-Parinacota, Chile. The samples were identified by agricultural engineer Andrés Huanca.

Essential Oil Extraction and Analysis

The essential oil (EO) was extracted from 300 g of *C. gilliesii* fresh leaves by hydrodistillation for 3 h in a Clevenger-type apparatus. The EO was dried over anhydrous sodium sulfate. Analysis of EO components was performed by gas chromatography and gas chromatography/mass spectrometry (GC/MS) using the instrumentation described below. Qualitative analysis was performed using a Thermo Scientific Trace GC Ultra linked to an ISQ quadrupole mass spectrometric detector with an integrated data system (Xcalibur 2.0, Thermo Fisher Scientific Inc., Waltham, MA, USA); quantitative

analysis was carried out using a Shimadzu GC-9A gas chromatograph fitted with an FID-9 detector (Shimadzu Corporation, Kyoto, Japan). The same capillary column (Rtx-5 MS, film thickness 0.25 µm, 60 m x 0.25 µm, Restek Corporation, Bellefonte, PA, USA) was used in both instruments. The operating conditions were as follows: on-column injection; injector temperature, 250º C; detector temperature, 280º C; carrier gas, He at 1.25 mL/min; oven temperature program: 40º C increase to 260º C at 4º C/min, and then 260º C for 5 min. The mass spectra were obtained at an ionization voltage of 70 eV. Recording conditions employed a scan time of 1.5 s and a mass range of 40 to 400 amu. Compound identification in the chromatographic profiles was achieved by comparison of their mass spectra with a library database (NIST11, NIST, Gaithersburg, MD, USA) and by comparison of their calculated retention indices with those reported in the literature (Adams, 2007) for the same type of column. ^{1}H-NMR spectra of the essential oil was obtained on a Bruker AVANCE 400 spectrometer (400 MHz) in $CDCl_3$, using the residual solvent peaks as internal standard.

RESULTS AND DISCUSSION

After fresh milling of *C. gilliesii* leaves (300 g), 0.45 g of EO was obtained (0.15%). The EO was analyzed by GC/MS (Table 1). Nine compounds were identified in the EO, corresponding to 99.06% of all detected compound. The monoterpenes pulegone (93.87%), isopulegone (3.07%) and menthofuran (0.54%) were the most abundant compounds in the EO. Identification was corroborated by studying the fragmentation patterns in the obtained mass spectra of the compound.

Table 1
Chemical composition of essential oil of *Clinopodium gilliesii* (Benth.) Kuntze

Compound	KI_{lit}[a]	KI_{exp}[b]	Concentration (%)[c]	Identification[d]
α-pinene	898-986	938	0.28	MS, KI, ST
sabinene	940-1001	983	0.27	MS, KI
β-pinene	947-1007	993	0.17	MS, KI, ST
limonene	1027-1031	1035	0.2	MS, KI, ST
eucalyptol	1001-1054	1040	0.32	MS, KI, ST
menthofuran	1142-1171	1174	0.54	MS, KI
Isopulegone	1155-1188	1188	3.07	MS, KI
α-terpineol	1140-1231	1202	0.34	MS, KI, ST
N.I.	-	1223	0.11	-
pulegone	1203-1258	1255	93.87	MS, KI

[a]Experimental Kovats index on an Rtx-5 column in reference to *n*-alkanes.
[b]Kovats index on a DB5 column in reference to *n*-alkanes from Adams, 2007.
[c]Peak areas relative to total peak area of identified compounds in the GC–MS chromatogram.
[d]MS, NIST, MS library and the literature; KI, Kovacs index; ST, authentic standard compound.

Additionally, using ^{1}H-NMR studies of the EO of *C. gilliesii* it was possible to identify pulegone, the assignment was performed using spectroscopic data published in the literature (Swigar & Silverstein, 1981).

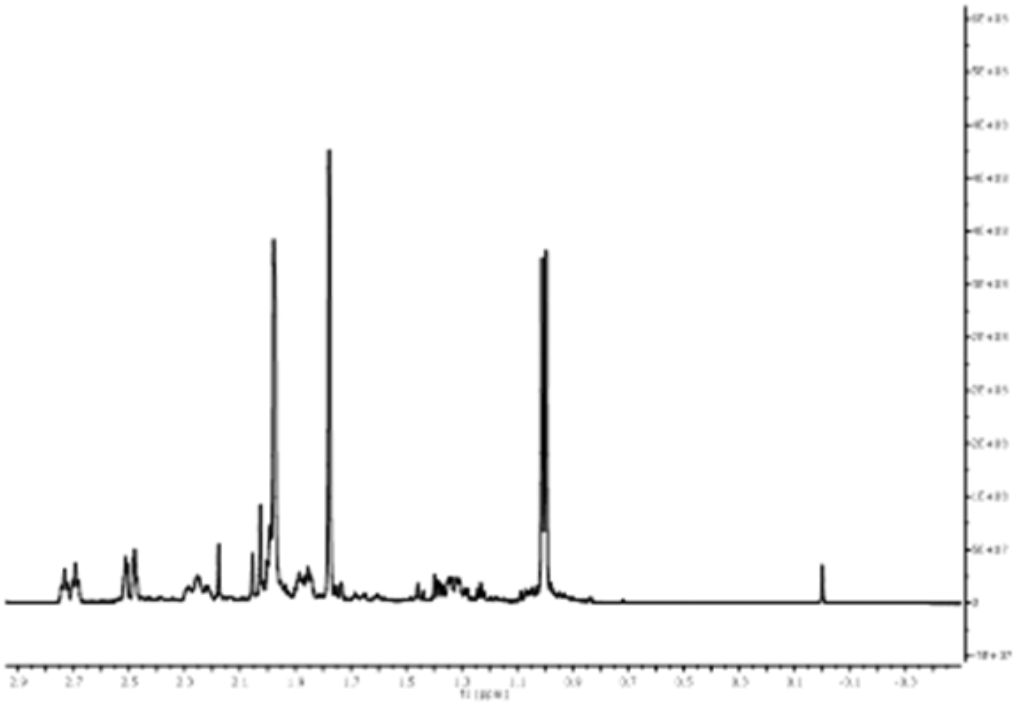

Figure 1
^{1}H-NMR spectrum of *Clinopodium gilliesii* essential oil

The amount of pulegone (93.87%) in the essential oil of leaves of *C. gilliesii*, collected in the Chilean highlands, showed remarkable differences with those reported by other authors. In studies carried out with samples collected in Argentina, the main monoterpenes in the oils corresponded to: piperitone, piperitenone, piperitenone oxide, *cis*-piperitenone epoxide, carvacrol and carvacryl acetate. Exceptionally and in < 20%, they are also found: (*E*)-Isocitral, pulegone, isopulegyl acetate, ρ-cymene and menthol. (De Iglesias *et al.,* 1978; Zygadlo *et al.,* 1993; Zygadlo & Grosso, 1995; Muschietti *et al.,* 1996; Viturro *et al.,* 2000; Viturro *et al.,* 2007; Luna *et al.,* 2008; Dambolena *et al.,* 2009; Lima *et al.,* 2011; Barbieri *et al.,* 2016).

The only exception is the essential oil composition of aerial parts of *Satureja parvifolia* (*C. gilliesii*) collected at flowering stage, Tafí del Valle, Tucumán, Argentina (1800 masl) infested with galls, containing 61.6% of pulegone (González *et al.,* 2010).

This remarkable difference on the essential oil composition should be seriously taken, particularly considering the huge amount of pulegone found in the essential oil of *C. gilliesii*. (+)-Pulegone is a *p*-menthane monoterpene ketone [*p*-menth-4(8)-en-3-one] found in essential oils from many mint species (e.g. *Minthostachys mollis* (Kunth) Griseb., *Minthostachys verticillata* (Griseb.) Epling *Hedeoma pulegioides* (L.) Pers. and *Mentha pulegium* L.). (Rojas & Usubillaga, 1995; Woolf, 1999; Escobar *et al.,* 2015). This monoterpene is considered hepatotoxic and potentially, carcinogenic in male and female mice, and female rats (Woolf, 1999).

In vitro pulegone is oxidized by cytochromes P450 to menthofuran which is then bioactivated to the reactive enonal, γ-ketoenal (Figure 2). The *in vivo* metabolism of pulegone is more complicated nevertheless; pulegone and menthofuran have been described to be oxidized by hepatic cytochromes P450 in humans, rats and mice to the electrophilic metabolite, γ-ketoenal, which are capable of covalent binding to cellular proteins, causing hepatic injury (Zhou *et al.,* 2007).

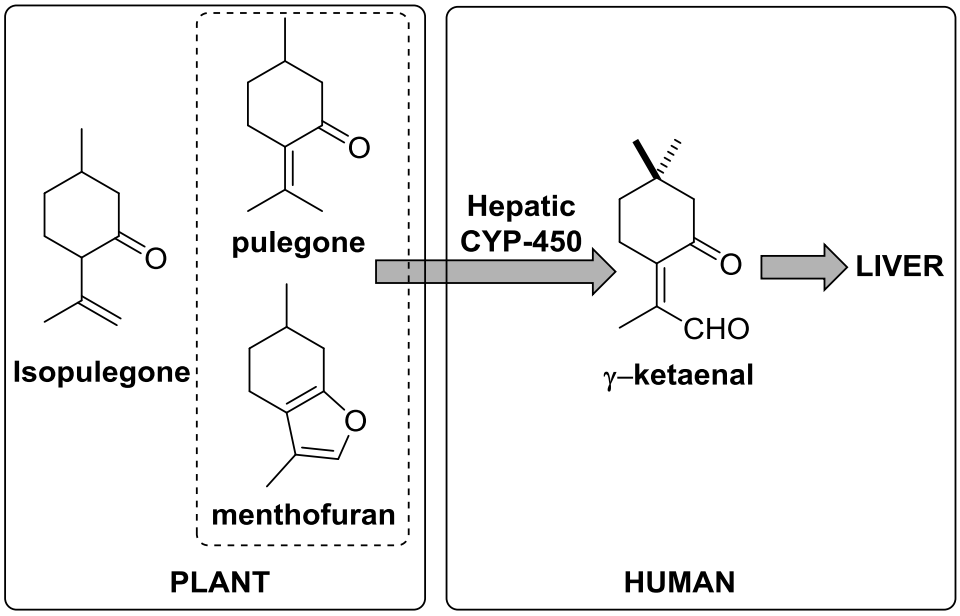

Figure 2
Metabolic transformation of main monoterpene presented in essential oil of *C. gilliesi*

In addition, the International Agency for Research on Cancer (IARC) has classified pulegone as a 2B carcinogen, possibly carcinogenic to humans. As a recommendation, the Committee on Herbal Medicinal Products (HMPC) has proposed that no-observed-adverse-effect level (NOAEL) is 0.75 mg/kg b.w. per day. Further, a safe daily dose for an adult of 60 kg body weight has been determined to be 45 mg (European Medicines Agency, 2016).

In the Chilean highlands, people suffering from stomach and respiratory problems use *C. gilliesii* as a medicinal plant. This practice involves the intake of 20 g infusions of *C. gilliesii* leaves daily (Kozel, 1991). Our results here indicate that one dry gram of *C. glilliesii* contains around 4.5 mg of pulegone, and that the amount ingested by drinking the infusion is around 90 mg per day. This value is twice higher than the recommended safe daily dose.

Although consumption of *C. gilliesii* as a medicinal plant may be a potential risk for human health, there are no studies in Chile about the potential increase in liver pathologies due to ingestion of pulegone. Additionally, there are no official recommendations or control of the plant commercialization in the markets and internet.

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