Colliguaja integerrima Gillies & Hook.

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Colliguaja integerrima Gillies & Hook. in its habitat (Comodoro Rivadavia, Chubut, Argentina). Photo: ML Flores and OL Córdoba

Abstract *Colliguaja integerrima* Gillies & Hook. (Euphorbiaceae) is an evergreen shrub native to Argentina and Chile, widely distributed in the Patagonia region. Tehuelches-Mapuches, the native people of Patagonia, have used this abundant plant to treat corns, warts and toothache and used the "colihuai" latex from the leaves and young branches, considered very poisonous, to poison their spearheads and arrowheads. Also, these were used as plasters for their analgesic properties. A decoction of the plant was used by Tehuelches for vaginal infections.

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Carbohydrates (polysaccharides with glucose, galactose, sulfate groups and pinitol), proteins, lipids (containing ricinoleic acid), flavonoids (datiscetin, kaempferol, pelargonidin and delfinidin), tannins, steroids and triterpenes (betulin) are the principal compounds. The chemical composition of this plant and the demonstrated antibacterial and antioxidant activities are evidences that could broadly support the traditional use. Methanolic and aqueous extracts evidenced important cytotoxicity against *Artemia salina*. The dichloromethane fraction, rich in betulin, generated 100% inhibition (at 100μg/ml) against promastigotes of *Leishmania donovani* (causal of visceral leishmaniasis). In view of its background in traditional medicine, this chapter reviews scientific studies on the chemical and biological properties of this species.

Keywords "Colihuai" · Euphorbiaceae · Tehuelches · Patagonia Argentina · Bioactive metabolites

1 Introduction

The Golfo San Jorge District (Patagonia Argentina) has a great vegetal diversity, mainly species typical of semi-desert and saline areas, with the ability to adapt to intense solar radiation and soils containing significant amounts of hydrocarbons.

Some species were used medicinally by aboriginal peoples, mainly by Tehuelches and Tehuelches-Mapuches. However, many of them are still less investigated from an integral perspective that allows validating their traditional uses.

Colliguaja integerrima Gillies & Hook. (Euphorbiaceae), is a shrub native to Argentina and Chile, widely distributed in the Golfo San Jorge District. This species was traditionally used to treat diseases such as calluses, warts and toothaches. Its very poisonous latex was used to poison spearheads and arrowheads.

The wide variety of already identified metabolites with demonstrated biological activities seem to be consistent with the traditional uses reported. The possible unexplored valuable properties of the species seem to call for further investigations.

2 Taxonomic Characteristics

Colliguaja integerrima Gillies & Hook. (syn.: *Colliguaja bridgesii* Müll. Arg.; The Plant List 2019), with the native name "colihuai", is a South American species belonging to the family Euphorbiaceae. This family, with around 220 genera and more than 7200 species, is found widely distributed in tropical and subtropical regions with largest centers in America and Africa. It is one of the largest and more

diverse families of flowering plants. The species ranked here are trees, shrubs or herbs, annual or perennial, in some cases woody, with or without latex. Various examples are found in the special literature on species of this family that are used in traditional medicine: many of these has been classified as toxic (Ragonese and Milano 1984).

The genus *Colliguaja* is a South American native (Chile and Argentina) and comprises five species: *C. brasiliensis* Klotzsch ex Baill, *C. dombeyana* A. Juss, *C. integerrima* Gillies & Hook., *C. odorifera* Molina and *C. salicifolia* Gillies & Hook. (The Plant List 2019).

Colliguaja integerrima was described by Gillies and Hook. and published in Botanical Miscellany in 1830 (The Plant List 2019). *Colliguaja* is a word of Mapuche origin ("colli" = reddish, "huai" = shrub), relating to the color of the reddish branches of the plant.

Ferreyra and Lorraine (2012) published a correction indicating that *Colliguaja integerrima* would mean "completely whole", in Mapuche language. Other common names are: in Spanish, "duraznillo", "duraznillo patagónico", "coliguay", "colihuai", "colihui", "colihuasi", "colliguay", "colliguaya", "coliguay del cerro", "lechón", "vegetal artillero"; Mapuche: "collihuai", "collihuayu"; Araucanian: "kolliwai", "collihuaye"; Tehuelche: "akanaiúwütr". The similarity of the fruit with small peaches ("duraznos" in Spanish) has earned it the popular name of "duraznillo" (Barboza et al. 2009; Desmarchelier and Alonso 2015).

3 Crude Drug Used

The main parts of the *Colliguaja integerrima* plant used in traditional medicine are the aerial organs (photo 1) and the latex exudate from the leaves and young branches, a white and sticky juice (de Mösbach 1992). These were used as plasters for analgesic properties (Ciampagna 2014).

4 Major Chemical Constituents and Bioactive Compounds

Carbohydrates, phenols and terpenes are the principal constituents of the aerial parts, fundamentally, they are present in the leaves. Latex contains phenols and terpenes. These chemical groups are related to medicinal properties. The following compounds were identified in the methanolic extract of aerial parts: condensed tannins, flavonoids (kaempferol, datiscetin, pelargonidin and delfinidin), hydroxycinnamic acids, predominantly synaptic acid. Delfinidin is a flavonoid with high antiperoxidative power, rare and has not been previously described for the genus *Colliguaja* (Pinto Vitorino et al. 2004a, b). Alcalde et al. (2005) described the presence of wax, palmitic alcohol and mono and di-palmitins in aerial parts. The main fatty acids identified were myristic, palmitic, palmitoleic and ricinoleic acids.

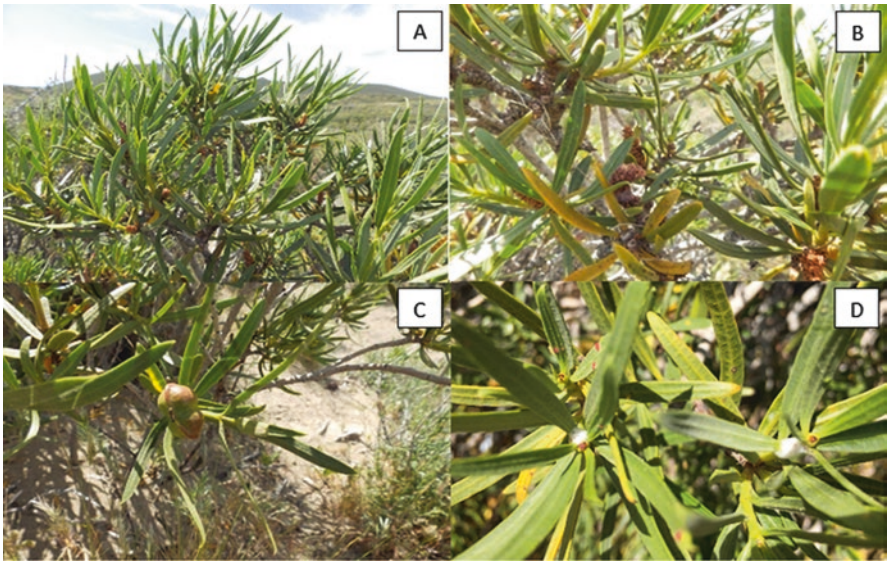

Photo 1 *Colliguaja integerrima* Gillies & Hook. (**a**) Plant detail. (**b**) Inflorescence. (**c**) Fruit. (**d**) Leaves with latex. (Photos: G Pinto Vitorino, ML Flores and OL Córdoba)

Gnecco et al. (1996) described for *Colliguaja integerrima* some hydrocarbons; the main is the *n*-heptaeicosane (C27) with 87% followed by C25, C29, C26, C31. Bittner et al. (2001) described that the whole plant presents lupeol, ursolic acid, oleanolic acid, β-sitosterol and carbohydrates.

The leaves of *C. integerrima* contain carbohydrates, proteins, lipids, flavonoids, tannins, steroids and triterpenes as principal compounds. Ethyl acetate fraction of the methanol extract from the leaves showed flavonoids and tannins as main metabolites, particularly kaempferol, datiscetin and myricetin, among others; in particular, datiscetin was described for the first time in this genus (Pinto Vitorino et al. 2004a, b, 2014). Dichloromethane fraction presented several terpenes, being betulin the main component (Quezada et al. 2015).

The principal polysaccharides obtained with aqueous extraction contained glucose followed by galactose, mannose, arabinose and rhamnose; sulfate groups are very important too. Also, in the leaves was found pinitol (*O*-methyl inositol) (Carrizo et al. 2018).

The inflorescences show the same metabolites groups that the leaves, except triterpenes, and larger amounts of proteins. The monosaccharides identified were galactose, mannose and xylose. The aqueous extract obtained at room temperature contains 7.7% of total sugars and 11.8% sulfate groups. In the aerial parts, the content of total sugars was 5.7% and sulfate groups 8.4% (Pinto Vitorino et al. 2004a, b).

The total phenolic content was 12.9%, both in aerial parts and in inflorescences, of that quantity the percentage of flavonoids corresponds to 6.2% and 18.2% respectively (Pinto Vitorino et al. 2004a, b).

The seeds of *C. integerrima* contain glucose, galactose, proteins, triterpenes and lipids. The total content of lipids obtained was 30.7% (Pinto Vitorino et al. 2004a, b). Malec et al. (1986) reported for seeds a 54.9% of crude oil (not refined). It is possible to assume that these variations could be due, between other factors, to the extraction technique applied, the time of sample collection, as well as to climatic and edaphic differences of the collection area (Pinto Vitorino et al. 2004a, b). *C. integerrima* seeds are a source of drying fixed oils. Riganti et al. (1947) and Malec et al. (1986) determined the chemical composition of fatty acids of the seeds. The major components were oleic, linoleic, linolenic and palmitic acid, and in less quantity were present myristic, stearic, arachidonic, behenic, and palmitoleic acid, with traces of eicosenoic and docosenoic acids. Cholesterol, campesterol, stigmasterol and sitosterol were found too. Ravetta et al. (1991) carried out a study on this species, in order to determine the production and oil content of *C. integerrima*. Proximal analysis of the seeds indicated a composition of 35% oil, with a content of 51% proteins, and a lysine quantity equal to 3.17 g/16 g N_2.

5 Morphological Description

Colliguaja integerrima is a native monoecious evergreen shrub, containing a milky white latex. It reaches 1–2 m height and 1.5 m high diameter. The branches are of reddish-brown color, erect and glabrous. The leaves are sessile, opposite, simple, leathery, linear up to 5 cm in length, obtuse and mucronated, of whole margins, with a prominent central rib of whitish hue (Riedemann et al. 2014). Unisexual flowers gathered in a single inflorescence. Flowers are lacking petals and sepals. Male flowers are terminal spikes of 2–3 cm in length and one or two female flowers are at the base of the male-spikes. The fruit is a capsule with 2 or 3 lobes (Ragonese and Milano 1984). Its flowering season occurs from September to November (Navas 1976). The vegetative growth occurs from September to February, the fruiting season occurs during March–April, and fruits are 3-seeded dry capsules (Navas 1976; Ravetta et al. 1991; Hoffman 1998). The fruits are capsules, strongly lignified, burst abruptly under the sunstroke, projecting the seeds (de Mösbach 1992).

6 Geographical Distribution

The genus *Colliguaja* is endemic in South America. It is represented by five species (Pinto Vitorino et al. 2014). *C. integerrima* is native to Argentina and Chile. In Argentina, it grows wild in the phytogeographical provinces of Patagonia (provinces of Mendoza, Neuquén, Chubut and Santa Cruz). In Central Patagonia, it is widely distributed in the Distrito Golfo San Jorge, zones where the species grows in a wild state (Ramírez 2002).

Ravetta et al. (1991) proposed that the domestication and cultivation of this species could be promising, especially in view of its adaptation to semi-deserts weather and its production of seed oil and by-products. Also, the production of hydrocarbon and petrochemical substitutes would increase interest in the species. However, due to its low economic value, there are no records of domestication experiments related to this species for commercial purposes. In the area of the Golfo San Jorge, Province of Chubut, and in order to recompose the vegetation cover before oil spills, they were carried out experiences with *C. integerrima* for recovery of the hydrocarbon through biodegradation *in situ*, land conditioning and plantations with shrub and tree species (Desmarchelier and Alonso 2015).

7 Ecological Requirements

C. integerrima inhabits the Patagonian steppe, where it grows in communities of different sizes. Usually in windy places with sandy, poor and rocky soil abundant in calcium carbonate; in sunny and "huaycos" (bad lands) zones, with precipitation of 100 mm/year and drought periods from 6 to 10 months. The individuals can survive at low temperatures (until −8 °C) and can tolerate the snow cover for several weeks, during the winter season (Quintana 2014; Gandullo et al. 2016).

There are also reports that indicate that *Colliguaja* species suffer a lower rate of defoliation caused by chewing insects, as compared to other shrub species in central Chile (Montenegro et al. 1980).

8 Traditional Use (Part(s) Used) and Common Knowledge

Tehuelches-Mapuches, the native people of Patagonia used this abundant shrub to treat corns, warts and to soothe the toothache (Ragonese and Milano 1984; Ravetta et al. 1991). The rural population of Patagonia used this plant to cure sheep mange, taking care that the leaves do not touch the eyes, as they could cause serious eye irritation. Pehuelches (Argentina) used "colihuai" latex, considered very poisonous, as a poison of their spearheads and arrowheads (Casamiquela 1999). Aerial parts rich in latex were used for medicinal purposes by Tehuelches. Also, these were used as plasters for their analgesic properties. The decoction of the plant was used for vaginal infections (de Mösbach 1992). Its internal use is dangerous because this plant is cited as toxic to cattle, sheep and horses (Ratera and Ratera 1980; Bittner et al. 2001).

9 Modern Medicine Based on Its Traditional Medicine Uses

Several uses in popular medicine have been supported by different studies *in vitro* about the active principles. However, *in vivo* conditions and clinical trials in humans would be required, mainly because it has been reported as a toxic plant.

Bhakuni et al. (1976), described antibacterial activity against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis* and *Streptococcus faecalis*. The ethyl acetate extract of the aerial parts of *C. integerrima* demonstrated inhibitory activity *in vitro* on nasopharyngeal cancer cell cultures KB human. Comparatively, the same extract of the related species *C. odorifera* demonstrated cell growth inhibitory activity of mouse lymphocytic leukemia (Bhakuni et al. 1976).

By another way, the latex obtained by the incision of the cortex of *C. integerrima* showed no proteolytic properties (Sequeiros et al. 2003).

Ethyl acetate fraction obtained from the methanolic extract of the leaves of *C. integerrima*, enriched in flavonoids, was active against *E. coli*, *S. aureus* and *P. aeruginosa*, with MIC values of 0.25, 0.50 and 0.25 mg/ml, respectively (Pinto Vitorino et al. 2014). The inhibition growth of the bacteria *Pseudomonas aeruginosa* has been relevant in this study. Despite improvements in antibiotic therapy, *P. aeruginosa* is intrinsically resistant to a great number of antimicrobial agents, frequently it is necessary to employ multiples classes of antimicrobial agents.

The antioxidant activity of the ethyl acetate fraction was very significant, with a concentration required to scavenge 50% of the free radicals (SC_{50}) of 5.7μg/ml. Also, against *Artemia salina,* it presented an LD_{50} = 77.3μg/ml. At high doses (1%), this fraction inhibited root length in a higher way, as vinblastine sulphate solution of 0.02% (Pinto Vitorino et al. 2014).

Dichloromethane fraction, rich in betulin, generated 100% inhibition (at 100μg/ml) against promastigotes of *Leishmania donovani* (causal of visceral leishmaniasis) (Quezada et al. 2015).

Álvarez and Borkowski (2007) described that the "collihuai" infusion contains permeation enhancers, substances used to promote the transdermal administration of drugs, which has significant advantages over other forms of administration, although they cause irritation (among other damages) in the skin.

10 Conclusions

The chemical composition of *Colliguaja integerrima*, and its proven antibacterial and antioxidant activity, are evidences that broadly support the traditional uses in conditions of toothache, which is frequently related to infection, inflammatory processes and ROS-induced tissue damage. The capacity of *C. integerrima* extracts to inhibit radicular growth and important antioxidant activity have been related to cytotoxicity and antitumor effects. These capabilities could justify the popular use

by the native peoples of Patagonia to treat corns and warts. All these results show the prospective medicinal potential of this species.

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