Cissampelos pareira L.

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Cissampelos pareira growing in Paso de la Patria, Corrientes, Argentina. Photos: BV Ricciardi Verrastro

Abstract *Cissampelos pareira* L. (Menispermaceae) is a widely used medicinal plant, also used to treat bites of venomous animals, particularly snakes. Ophidian accidents are a serious public health problem in Argentina where the *Bothrops*

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genus of snakes is responsible for 97% of these accidents, peculiarly *B. diporus* ("yarará chica") responsible for 80% of them. In the northeast of the country (Corrientes Province), *C. pareira* is commonly used against the venom of *B. diporus*; its use is described in almost all-ethnobotanical literature of the countries where the plant grows. *In vitro* and *in vivo* antivenom activities of *C. pareira* extracts from plants collected at two different phytogeographic regions of Corrientes (Argentina) were evaluated against *B. diporus* venom. The seasonal influence on the chemical composition of extracts was also studied to determine the associated range of variability and its influence on the antivenom activity. Besides, an evaluation of the chemical composition of the volatile extract was performed to assess its chemical stability. These results support the ethnopharmacological use of this species. Moreover, the presented data demonstrate that certain flavonoids may mitigate some of the venom-induced local tissue damages.

Keywords Antivenom activity · Phytochemical · *In vitro* and *in vivo* activities · "Ka'apevá"

1 Introduction

Production of secondary metabolites by plants represents an adaptive capacity to environmental challenges and the changing of growth conditions. For centuries, medicinal plants' usage evolved as a result of the many years of humanity struggle against illnesses and its learning to pursue drugs in different parts of the plants. In time, many plants have been used as antidotes for snake envenomations (Inoue et al. 2019). Usually, antivenoms are hyper immune sera collected from animals which bind and inactivate venom components. Over the years many attempts have been made for the development of snake venom antagonists especially from plant sources, so many reports can be found in the literature related to the use of medicinal plants against snakebite by the different ethnic communities throughout the world, especially in rural parts from tropical and subtropical countries. Some of the plant extracts were found to have a potency as antivenom *in vitro* but failed to show venom neutralizing ability *in vivo* (Gupta and Peshin 2012).

Generally, local effects of a snakebite occur in the first 10–30 min; and there may be numbness around the bite with bleeding, or a purpuric rash, and/or necrosis or gangrene (De la O Cavazos et al. 2012). These local reactions are not effectively neutralized by conventional antivenom serum therapy. In severe cases, local effects of envenoming may lead to permanent tissue loss, disability, or amputation (Gutiérrez 2002).

The lack of medical infrastructure in the rural areas, human ignorance, the side effects of animal-based antivenoms are issues that explain the necessity to develop alternative therapies, so that snake bite investigators engaged in finding out the scientific basis of certain plants' use as antiophidian ethnomedicine.

The current chapter discusses the pharmacognostic characteristics of *Cissampelos pareira* L. (Menispermaceae) with the aim of providing useful information for its correct identity. However, the information is focused on scientifically explaining the ethnobotanical use of *C. pareira* against venom of *Bothrops diporus* in Northeast Argentina by identifying those compounds responsible for the alexiteric activity.

2 Taxonomic Characteristics

The name of the *Cissampelos* genus derives from the Greek *kissos* which means ivy and *ampelos*, vine, referring to its fruits arranged in form of clusters. In Argentina, the species is commonly known as "ka'apevá", "ka'á-pevá", "ysypó-morotí", "caá-pebá", "zarza", "pareira brava" or "mil hombres" (Ricciardi et al. 1996).

C. pareira is a climbing plant with alternating simple leaves that are usually zygomorphic in this genus, usually small, greenish, or white (Photo 1). The fruit is an aggregate of drupes.

Synonymous: *Cissampelos pareira* L. var. *australis* (A.St.-Hil.) Diels, *Cissampelos pareira* L. var. *caapeba* (L.) Eichler, *Cissampelos pareira* L. var. *gardneri* Diels, *Cissampelos pareira* L. var. *tamoides* (Willd. ex DC.) Diels, *Cissampelos auriculata* Miers, *Cissampelos hederacea* Miers, *Cissampelos monoica* A. St.-Hil., *Cissampelos pareira* L. var. *monoica* (A.St.-Hil.) Eichler, *Cissampelos australis* A. St.-Hil., *Cissampelos litoralis* A. St.-Hil., *Cissampelos pareira* L. *f. reniformis* Chodat & Hassl., *Cissampelos pareira* L. *f. emarginato-mucronata* Chodat & Hassl., *Cissampelos caapeba* L, among others (Zuloaga and Belgrano 2014; The Plant List 2019).

Photo 1 Aerial parts of *Cissampelos pareira*. (Photo: B Ricciardi Verrastro)

3 Crude Drug Used

C. pareira leaves and roots are used in aqueous infusions (Leonti et al. 2001; Jain et al. 2005; Namsa et al. 2011; Heinrich et al. 2014). In certain circumstances leaves are also applied topically or macerated in wine (Abbasi et al. 2010; Haq et al. 2011).

4 Major Chemical Constituents and Bioactive Compounds

Despite the large amount of information on *Cissampelos pareira* phytochemistry, this review focuses on the results obtained with the study of plant extracts on local effects of *Bothrops diporus* envenomation.

Volatile Fraction Semwal et al. (2014) only cite the presence of thymol in roots. By simultaneous distillation extraction (SDE), we found the volatile extracts showed the following chemical composition by GC-MS (Table 1) (full data not published).

The volatile extract from aerial parts was characterized by β-pinene (23.2%), limonene (22.6%), α-pinene (8.8%), myrcene (6.3%) and (*E*)-β-ocimene (6.3%). While in roots, the main volatile compounds were limonene (34.8%), stearic acid (14.2%), myrcene (4.9%), methyl palmitate (4.1%), α-pinene (3.3%) and carvone (3.4%). On the other hand, the presence of oxygenated monoterpenes and fatty acids in the volatile fraction of the roots was remarkable (Table 1).

Nonvolatile Fraction Extracts of *C. pareira* have been widely studied in different countries, and their components are divided into two main chemical groups: alkaloids (Table 2) and non-alkaloids (Table 3).

Some components have biological properties: tetrandrine (analgesic, antipyretic, anti- inflammatory, cardioactive, and hypotensive effects), pareirubines A and B (antileukemic), alkaloids (febrifuges and curarizes), berberine (hypotensive, antimicrobial, and antifungal effects) (Sánchez-Medina et al. 2001) and cissampelin (muscle relaxant) (Semwal et al. 2014).

Table 1 (%) volatile compounds families

Compound family	% aerial parts	% roots
Monoterpenes hydrocarbons	76.4	66.5
Oxygenated monoterpenes	2.8	11.9
Sesquiterpenes hydrocarbons	4.0	0.6
Oxygenated sesquiterpenes	0.1	0
Nor isoprenoids	1.9	0.1
Fatty acids	0	24.7
Fatty acid esters	0	4.0
Others	0.5	0.3
Not identified	17.1	3.7

Table 2 Alkaloid components from *C. pareira*

Alkaloid	Organ	Reference
Pelosine (bisbenzylisoquinoline)	Roots	Wiggers (1840)
Hayatine, (−)-curine	Roots	Scholtz (1896)
Hayatinine	Whole plant	Bhattacharji et al. (1952, 1956, 1962), Bhatnagar et al. (1967), Bhatnagar and Popli (1967), and Haynes et al. (1966)
Isochondodendrine, cissamperine	Roots; fruits; whole plant	Kupchan et al. (1960, 1965, 1966)
Hayatine, berberine, curine, isochondodendrine	Roots	Boissier et al. (1965)
Cissamine hydrochloride, cyclanoline, isochondodendrine, dicetrin	Roots	Anwer et al. (1968)
Dehydrodicentrine, cicleanine, insularine, berberine, hayatidine	Roots	Dwuma-Badu et al. (1975)
Isochondodendrine, cicleanine, sepeerine	Whole plant	Bhakuni et al. (1987)
Warifteine, methyl-warifteine	Leaves	Aguirre-Galvis (1988)
Laudanosine, nuciferine, bulbocarpine, corituberine, magniflorine hydrohloride	Leaves and stems	Ahmad et al. (1992)
Pareirubrines A and B, grandirubrine, isoimerubtine	Whole plant	Morita et al. (1993a, b)
Azafluoranthene, norimeluteine, norruffscine	Whole plant	Morita et al. (1993c)
Pareitropone	Whole plant	Morita et al. (1995)
Berberine, reserpine, cissampeline (pelosine)	Roots	Sharma et al. (2004), Stepp (2004), Bafna and Mishra (2010)
Magnoflorine, magnocurarine	Roots	Bala et al. (2017)

Table 3 Non alkaloid components from *C. pareira*

Non-alkaloid component	Active organ	References
Quercitol	Roots	Srivastava (1956), Chowdury (1972), Dwuma-Badu et al. (1975)
Sterols	Aerial parts	Ramírez et al. (2003)
Cissampeloflavone	Leaves	Singthong et al. (2005)
Chalcone flavone dimer	Whole plants	Amresh et al. (2007a)
Pectins	Whole plants	Ramasubramaniaraja and Badu (2010)
Galacturonic acid (70–75%) and neutral sugars	Leaves	Vardhanabhuti and Ikeda (2006)

For native materials from Paso de la Patria (Corrientes, Argentina) of this species, whose ethanolic extracts showed to be active as alexiteric, the phytochemical analysis showed the stability of phenols and anthraquinones in different vegetative states of the species, while alkaloids were only found in extracts from autumn and saponins in extracts obtained from spring and summer. After bio-guided fractionation of the ethanol extract of aerial parts, following alexiteric activity, the phytochemical analysis resulted in presence of alkaloids, phenols and flavonoids in the most alexiteric active fraction. Possibly the alkaloids and flavonoids have not been identified in the whole extract due to a dilution phenomenon. In addition, steroids would not be related to the biological activity of the extracts, due to their presence in the less active fractions. The presence of polyhydroxylated flavonoids was detected due to its UV spectra by HPLC DAD, in the active fraction analysis. The structural elucidation of flavonoids was carried out by UPLC-MS, identifying quercetin-3-*O*-sophoroside [quercetin-3-*O*-β-D-glucosyl-(1→2)-β-D-glucoside], naringenin-7-*O*-β-D-glucoside, eriodictyol-7-*O*-β-D-glucoside, galangin-7-glucoside, and baicalein-7-*O*-glucoside (oroxin A) (Ricciardi-Verrastro et al. 2018).

5 Morphological Description

C. pareira is a perennial climbing plant, branched, striate, 2–5 m high with a thickened root and pubescent or sub-glabrous. Leaves are peltate and alternate, 3.8 to 14 cm in diameter orbicular or reniform often slightly broader than long, cordate or sometimes truncate at the base. The plant is dioecious with flowers arranged in bunches green, white or yellow; the staminate (male) has 4 free sepals and 4 fused petals; pistillate (female) flowers have 1 sepal, 1 petal, and a solitary carpel. The fruits are small red or yellow drupes (Don 1831; Rhodes 1975; Stevens 2001; Tamaio et al. 2010; Singh et al. 2013a).

6 Geographical Distribution

Cissampelos is one of 70 genera belonging to the Menispermaceae family established by Linne in 1753 in the first edition of the *Species Plantarum* (Granda-Lorenzo and Fuentes-Fiallo 1991). It has a global distribution throughout the five continents, in regions of low altitude rainforests (up to 2100 m.a.s.l.) (Singh et al. 2013a).

In Argentina, there are 3 genera belonging to the Menispermaceae family: *Cissampelos*, *Hyperbaena*, and *Odontocaraya* (Zuloaga and Belgrano 2014). Their geographical distribution comprises the Argentinean provinces of Catamarca, Chaco, Corrientes, Formosa, Jujuy, Misiones, Salta, Santa Fe, Tucumán, Santiago del Estero for *Cissampelos* species, while *Hyperbaena* genus is located in Chaco and Formosa Provinces and *Odontocaraya* species have been reported for Corrientes,

Misiones, Chaco, Formosa, Misiones, Salta and Tucumán (Zuloaga and Belgrano 2014).

7 Ecological Requirements

Cissampelos pareira L. has worldwide distribution, occurring in tropical and subtropical regions of the Americas, Africa and Asia (Ortiz 2001). In Brazil, it is encountered from Caatinga, Atlantic Forest and Amazon forest. In Africa, this species occurs in subtropical forest, savannah, deciduous shrubs, often persisting in cleared land and plantations, also in secondary vegetation and near rock outcrops. It is the most popular species of *Cissampelos* not only for its wide distribution, but mainly because its leaves and roots are widely used as medicinal.

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

The popular uses of *C. pareira* are mentioned in almost all ethnobotanical literature from countries where the plant grows, including South America, Asia, and Africa. As per traditional knowledge, it is used as a carminative, febrifuge, for liver disorders, constipation, menstrual pain, colic, and rheumatism. It also has been used for cough, delirium, madness, epilepsy, seizures, as antiplasmodial, stimulant, sedative, analgesic, antioxidant, tonic and narcotic. (Gessler et al. 1994, 1995; Sudarsanam and Prasad 1995; Taylor 1996; Antoun et al. 2001; Leonti et al. 2001; Galicia et al. 2002; Kakrani and Saluja 2002; Rajan et al. 2002, 2003; Sharma et al. 2004; Chhetri et al. 2005; Jain et al. 2005; Kufer et al. 2005; Kumar et al. 2006; Bora et al. 2007; Mendes and Carlini 2007; Muthaura et al. 2007; Pattanaik et al. 2008; Rukunga et al. 2009; Abbasi et al. 2010; Ramasubramaniaraja and Badu 2010; Giorgetti et al. 2007; Gupta et al. 2011; Haq et al. 2011; Nagarajan et al. 2011; Namsa et al. 2011; Samanta and Bhattacharya 2011; Basha and Sudarsanam 2012; Kaur et al. 2012; Sharma et al. 2012; Singh et al. 2013b; Semwal et al. 2014; Heinrich et al. 2014).

According to ethnopharmacological surveys, decoctions of leaves and roots of *C. pareira*, as well as aqueous or alcoholic infusions, are traditionally used against ophidian venom (alexiteric plants: those which can relieve one or more complex symptoms such as pain, bleeding, inflammation, infection or even the same poisoning). In the Northeast of Argentina (Ricciardi et al. 1996), Paraguay (Jolis 1972; Manfred 1977; Montenegro 1979; González-Torres 2013) and many tropical countries, root decoction in water is used to treat snake bites and other poisonous animals (Morton 1981). In Mexico, the whole plant is used against snake venom (Ramos-Hernández et al. 2007) and its use also extends to Amazon (Ecuador and Peru) and Central America (Barranco-Pérez 2010). In India, root decoction or the whole plant (Chakraborty and Bhattacharjee 2006; Jabeen et al. 2009; Sankaranarayanan et al.

2010; Dey and De 2012); in Pakistan also use leaves or the entire plant in poultices applied on the bite site (Butt et al. 2015).

A 50% hydroalcoholic roots extract (2 g/kg) administered orally did not present acute or subacute toxicity (Amresh et al. 2008). Wipawee and Jintanaporn (2012), studied the acute toxicity of a 50% hydroalcoholic extract obtained from a commercial mixture of *C. pareira* and *Anethum graveolens* (1:5). Up to 5000 mg/kg body weight did not cause death or toxic rat symptoms. According to the guidelines of the Organization for Economic Cooperation and Development (OECD) for acute oral toxicity, an LD_{50} of 2000 mg/kg body weight is classified as unclassified and, therefore, the product is considered safe.

Piero et al. (2015) performed a histopathological analysis of liver, kidney, heart, spleen, brain, lungs, eyes and testicles of albino mice Swiss White after administration of aqueous extract of *C. pareira* leaves (1 g/kg body weight) for 28 days. When the administration was oral, no histopathological changes were evidenced in any of the organs analyzed. However, when the administration was intraperitoneal, changes in the liver were observed, with proliferation of fibrous tissue in the serous layer and presence of mixed inflammatory cells, which would be indicative of peritonitis; and in the spleen, a reduction in cell density of lymphoid follicles.

9 Modern Medicine Based on Its Traditional Medicine Uses

Some of the pharmacological properties of *Cissampelos pareira* have been scientifically investigated by different researchers, as shown in Table 4.

There are many citations on the alexiteric activity scientifically validated for *C. pareira*. Badilla et al. (2008) in Costa Rica, reported antihemorrhagic and antiproteolytic activity from an aqueous infusion of the entire plant against *B. asper* venom. Saravia-Otten et al. (2015) studied the ethanolic extracts of roots collected in Guatemala against *B. asper*; finding antiproteolytic activity but this extract failed to inhibit the hemolytic or coagulant activities of the venom. In Argentina, as above mentioned, the *Bothrops* species is responsible for 97% of the ophidian accidents, being the species *B. diporus* responsible for 80% of them. In order to scientifically evaluate this plant application, the *in vitro* and *in vivo* antivenom activities of *C. pareira* extracts were evaluated against *B. diporus* venom, with a focus on the local effects associated with envenoming.

The seasonal influence on the chemical composition of the active extracts was also studied; in order determine the associated range of variability and its influence on the antivenom activity. The research was conducted using aerial parts (leaves, flowers, tender stems) and roots of *Cissampelos pareira* collected from two different phytogeographic regions of Corrientes (Argentina): Paso de la Patria (PP) and Lomas de Vallejos (LV). In addition, to perform a seasonal analysis and to evaluate the metabolic stability, material was collected at three different growth stages.

In vivo and *in vitro* antisnake venom activities were tested, and a bio-guided chromatographic separation was performed in order to determine the active

Table 4 Biological activities of *C. pareira* scientifically validated

Biological activity	Responsible extracts	References
Antidiarrheal	Hydroalcoholic, roots	Amresh et al. (2004)
Anti-inflammatory	Hydroalcoholic 50%, roots; ethanolic	Amresh et al. (2007a, 2007b)
Analgesic and antipyretic	Ethanolic	Reza et al. (2014)
Antifertility	Methanolic and water, leaves	Ganguly et al. (2007, 2018) and Ampa et al. (2010)
Anthelmintic	Aqueous aerial parts	Padmani et al. (2012)
Antioxidant	Hydroalcoholic 50%, roots; alcoholic and ethyl acetate	Amresh et al. (2007c) and Gul et al. (2016)
Hepatoprotective	Hydroalcoholic 50%, roots	Surendran et al. (2011)
About memory and learning	Hydroalcoholic 50%, roots	Pramodinee et al. (2011)
Anxiolytic	Hydroethanolic 70%, leaves; alkaloids (berberine), terpenoids and phenolic compounds	Priyanka (2013)
Cardioprotective	Ethanolic roots; alkaloids (berberine); flavonoids	Patnaik et al. (1973), De Freitas et al. (1996), Amresh et al. (2007a) and Singh et al. (2013b)
Immunomodulatory	Methanolic, roots; *bis*-benzyl isoquinoline and berberine alkaloids	Bafna and Mishra (2010)
Antitumor	Protein and polysaccharide extract; methanolic extract	Meng et al. (2002) and Thavamani et al. (2014)
Antileukemic	Pareirubrins A and B; grandirubrins and isoimeribrin: Alkaloids	Morita et al. (1993a)
Anticancer	Hexane extract; oleanolic and oleic acids	Bala et al. (2014)
Gastric cancer protector	Hydroalcoholic 50%, roots	Amresh et al. (2007d)
Hypoglycemic	Water, leaves	Kuldeep et al. (2013)and Piero et al. (2015)
Antidengue	Ethanolic	Sood et al. (2015)
Simile curare	Hayatinine methyl hydrochloric	Basu (1970) and Bhatnagar and Popli (1967)
Nephroprotective	Hydroalcoholic of whole plant	Danduga et al. (2015)
Antiurolithic	Ethanolic, leaves	Babu et al. (2014)
Diuretic	Ethanolic, roots	Sayana et al. (2011)

chemicals involved. The fractions obtained were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and the chemical profile of the most active constituent was analyzed by ultra-high-performance liquid chromatography coupled to quadrupole/high-resolution mass spectrometry (Q-Orbitrap). (UHPLC-MS). The extracts analyzed showed significant differences in the modification of the venom band pattern of *B. diporus*. These differences depended on their vegetative state (season in which they were collected), on edaphological factors

(geographic region of collection) and also on the constituent organ of the plant (roots / aerial parts). In general, aerial parts collected in PP were more active than those collected in LV. However, in the LV, roots were more active.

Respect the vegetative state of the species in summer (flowering stage) the extracts of aerial parts were more active than in spring and autumn. The polar extract (ethanolic) showed greater activity than the aqueous and hexane. When results were analyzed, the alcoholic extract was found to be the most active. The bio-guided fractionation allowed selection one fraction to be analyzed by UHPLC-MS in order to identify the components responsible for the activities found; this study identified five possible flavonoids (Ricciardi-Verrastro et al. 2018).

The entire work on the activity of *C. pareira* against the venom of *B. diporus* allowed to confirm that this species possesses inhibitory effects in both *in vitro* and *in vivo* models. Moreover, these data demonstrate that certain flavonoids may mitigate some of the venom-induced local tissue damage. Considering the results of *C. pareira* from other regions of the world, these results are consistent with those obtained by Saravia-Otten et al. (2015) in Guatemala, who also found no activity in the ethanolic root extracts for *B. asper* venom.

The most alexiteric active fraction found was used to prepare a heat-sensitive gel formulation (1% plant extract, 10% ethanol 96°, 10% DMS, 80% poloxamer) and BALB/c mice were used *in vivo* tests to evaluate the ability to neutralize the local effect of *B. diporus* venom. Histopathological analysis showed differences between groups with and without dermal application. Group treated with heat-sensitive gel showed areas with absence of epidermis with mild inflammatory infiltrate and neutrophilic absence of hemorrhage, while the group without heat-sensitive gel treatment evidenced dermonecrosis and intense bleeding with inflammatory infiltrate. After 3 days of gel application, it was possible to obtain a remarkable enhancement in tissue renewal, demonstrating the activity and usefulness of the preparation. Even when it is essential to improve the permeation of the bioactive compounds, the formulation seems to be a valid option for local treatment (Ricciardi-Verrastro et al. 2016).

10 Conclusions

Investigations into the activity of *C. pareira* against the *B. diporus* venom confirmed that this species possesses inhibitory effects in both *in vitro* and *in vivo* models. The screening of the alexiteric activity by SDS-PAGE showed that plant material collected in different geographical regions can present different activity levels, indicating probable secondary metabolism expression differences. The aerial parts were more active than roots in experiments where a total clearance of the venom bands was observed after treatment with the ethanolic extracts of aerial parts collected in summer by SDS-PAGE. Considering that the snake bites occur in a higher incidence in persons and moderately high incidence in children, the verification of this property is particularly important (Chippaux 1998). Although local effects of snake bites

appear in the first 10–30 mins, these local reactions are usually not effectively neutralized by conventional antivenom serum therapy (Lomonte et al. 1994; Ávila-Agüero et al. 2001). In severe cases, the local effects of poisoning may lead to permanent tissue loss, disability, or amputation (Gutiérrez 2002).

The results presented show that flavonoids isolated from this species can mitigate part of the local tissue damages induced by venom. Toxicological studies should be continued with the aim to use this crude drug as an adjuvant in cases of snake accidents.

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