



## Pharmacobotany of Two *Valeriana* Species (Valerianaceae) of Argentinian Patagonia Known as “Ñancolahuen”

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**SUMMARY.** The genus *Valeriana* (Valerianaceae) with about 250 species is distributed worldwide except in Oceania. *Valeriana officinalis* is the most known and used species. Forty-nine species grow in Argentina, nineteen of them in the Patagonia region, including *Valeriana carnosa* and *Valeriana clarionifolia*, known as “ñancolahuen” and used in traditional medicine. The objective is to provide anatomical data to differentiate subterranean organs of *V. carnosa* and *V. clarionifolia*, in the condition that are marketed. Sectioning techniques, macerations and powdered material analysis were performed. *V. carnosa* showed a primary pentarch aktinostele root, pith in the secondary structure, and a rhizome with anomalous structure, whereas, *V. clarionifolia* has no rhizome and showed a protostele as a primary root structure and a secondary structure without pith. In the maceration, the rhizome of *V. carnosa* presented cork with irregular polygonal cells with acute and obtuse angles while, in *V. clarionifolia* rectangular cork cells with right angles were observed. Starch grains are simple, spherical in *V. carnosa* and polyhedral in *V. clarionifolia*. This study provides unpublished data to differentiate anatomically the two species of “ñancolahuen”, and discern between them in commercial samples.

**RESUMEN.** El género *Valeriana* (Valerianaceae) contiene cerca de 250 especies distribuidas en todo el mundo, excepto en Oceanía. *Valeriana officinalis* es la más conocida y utilizada. En Argentina crecen 49 especies, 19 de ellas en la región de la Patagonia, incluyendo *Valeriana carnosa* y *Valeriana clarionifolia*, conocidas como “ñancolahuen” que se utilizan en medicina tradicional. El objetivo del estudio es proporcionar datos anatómicos para diferenciar los órganos subterráneos de *V. carnosa* y *V. clarionifolia*, en las condiciones en que se comercializan. Se realizaron técnicas de seccionado, maceraciones y análisis de materiales en polvo. *V. carnosa* mostró una raíz primaria actinostela pentarca, médula en la estructura secundaria y un rizoma con estructura anómala, mientras que *V. clarionifolia* no tiene rizoma y mostró una protostela como estructura de la raíz primaria y una estructura secundaria sin médula. En la maceración, el rizoma de *V. carnosa* presentó súber con células poligonales irregulares con ángulos agudos y obtusos, mientras que en *V. clarionifolia* se observaron células rectangulares con ángulos rectos. Los granos de almidón son simples esféricos en *V. carnosa* y poliédricos en *V. clarionifolia*. Este estudio aporta datos inéditos para diferenciar anatómicamente las dos especies de “ñancolahuen” y discernir entre ellas en las muestras comerciales.

### INTRODUCTION

The genus *Valeriana* (Valerianaceae) has approximately 250 species worldwide distribution, with the exception of Oceania. Forty-nine grow in Argentina, nineteen of which are in the Patagonia region<sup>1-3</sup>. Sedative, anxiolytic, hypnotic and antispasmodic properties have been attributed historically, in traditional medicine, to the roots and rhizomes of several species of the genus, standing among others: *V. officinalis* L.:

“Valerian”, *V. wallichii* DC.: “Valerian of India”, and *V. edulis* Nutt.: “Mexican Valerian”<sup>4</sup>.

In Argentina, the species used by traditional medicine in the Patagonia region are *V. carnosa* Sm., *V. clarionifolia* Phil., both known as “ñancolahuen”, and *V. laphatifolia* Valh. called “Hualguinque”. In others regions of the country *V. effusa* Griseb., *V. ferax* (Griseb.) Höck. and *V. polybotrya* (Griseb.) Höck., have also traditional used<sup>5</sup>. Argentinian Patagonia region has

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many plants used in traditional aboriginal medicine, mainly by the Mapuche ethnicity. The medicinal uses are transferred through generations by oral and cultural transmission of information up to the present.

Among the various plants used, two species of *Valeriana* stand out. These are known as “ñancolahuen” and their subterranean organs are used to treat ailments of the lungs, stomach, liver, kidneys, waist pain and back pain, and for decay <sup>6</sup>. They are also used as analgesic and sedative to treat rheumatism <sup>7</sup>, to treat rheumatism and insomnia <sup>8,9</sup>, and for disorders related to heart and bladder <sup>10</sup>. Both “ñancolahuen” species are sold in health food stores and pharmacies as herbal medicines.

Usually medicinal plants are offered at different degrees of fragmentation and only under their common name, on that the botanical identification of species is difficult. In this paper, the aim is to provide anatomical data of subterranean organs (roots and rhizomes) to differentiate *V. carnososa* and *V. clarionifolia* in the condition in which they are marketed. Up to now many *Valeriana* species used in traditional medicine do not have a description of their anatomical structure. Therefore, their authentication is hampered in quality control works, where the identification of the species is indicated followed by a question mark <sup>11</sup>.

## MATERIALS AND METHODS

### *Plant material*

The studies were performed on the following materials: a) roots and rhizomes of specimens collected on trips to Patagonia, b) herbarium specimens from Institute of Biological Resources: BAB. The reference materials listed below, as well as permanent histological preparations obtained are deposited in the Herbarium of the Institute of Biological Resources (BAB) and Pharmacobotany Chair, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, respectively. The material was identified with keys according to Borsini<sup>12</sup> and the nomenclature of species was recorded according to Kutschker <sup>2</sup>.

#### *Valeriana carnososa*

Argentina. - Río Negro. Dpto. Bariloche: cerro Otto, ladera de rocas del lado NW de la aerosilla del cerro. Elev. 1100 m. 9-12-2008. Leg. R.H. Fortunato et al. 9447 (BAB); El Bolsón: alrededores del mirador del cerro Piltriquitron. Elev. 1200 m., 18-III-2010. Leg. H.G. Bach 415

(BAB); - Santa Cruz. Dpto. Lago Buenos Aires: orilla del Río Ceballos. 29-XI-1990. M.N. Correa et al. 10343 (BAB); - Tierra Del Fuego. Dpto. Río Grande. San Sebastián: borde de playa sobre la Bahía San Sebastián. 29-I-1995. Leg. R.H. Fortunato & M. Elechosa 4895 (BAB).

#### *Valeriana clarionifolia*

Argentina. - Chubut. Dpto. Futaleufú: frente al lago Futalauquen. 2-12-1990. Leg. M.N. Correa et al. 10441(BAB); - Neuquén. Dpto. Los Lagos: Ruta 23, 5 Km al E. del cruce con RN 231 en dirección a Pilcaniyeu. Elev. 900 m. 8-12-2008. Leg. R.H. Fortunato et al. 9433 (BAB); - Río Negro. Dpto. Pilcaniyeu: 2-3 Km. al NW de la pista del Aeropuerto, frente a la torre de aviación. Elev. 700 m. 6-12-2008. Leg. R.H. Fortunato et al. 9381 (BAB); - Santa Cruz. Dpto. Lago Buenos Aires: camino al río Jeinemen RP 250. 28-12-1990. Leg. M.N. Correa et al. 10322 (BAB).

## *Methodology*

Sectioning and coloring techniques, macerations and starch micrometry were performed on the roots and rhizomes. Cross sections were obtained by hand and with a Reichert sliding microtome. The obtained sections were stained with Safranin-Fast Green according to standard techniques <sup>13,14</sup>, and mounted with Canada balsam.

The maceration of organs consisted of taking small portions of the material to be examined, adding 5 % NaOH, allowing to boil for 10 min, and then washing with water and storing in ethanol 70° <sup>15,16</sup>.

For starch grains analysis, portions of roots and rhizomes were reduced to powder and observed under polarized light. Measurements of the starch grains were carried out with a calibrated micrometer eyepiece 10x PL Carl Zeiss.

Microscopic observations and photomicrographs were made with a Zeiss Axiolab MC 80 DX photomicroscope. The illustrations of the specimens were made by a scientific illustrator and the symbolism of Metcalfé and Chalk was used in the scheme of the cross sections <sup>17</sup>.

## RESULTS

### *Valeriana carnososa*

#### *Macroscopy*

The rhizome, greatly developed, is 2-6 cm in diameter and 60 cm in length; it has buds and determinate growth (simpodial). The surface is dark brown and has linear and / or helical stria-

tions, deeply furrowed. The secondary roots, 0.3-0.6 cm in diameter, are brown, striated, without furrows and have no buds (Fig. 1A; Fig 2A).

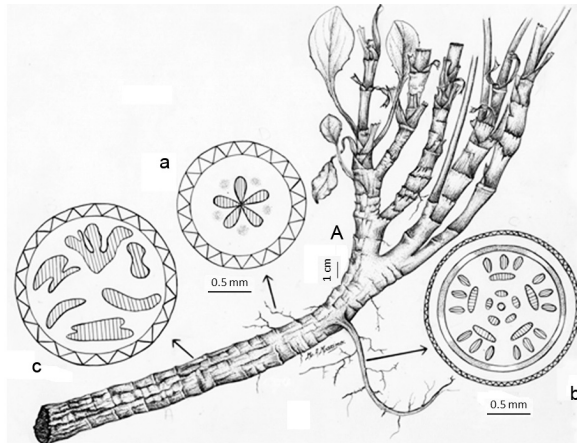
**Microscopy**

Cross sections of the primary roots showed a

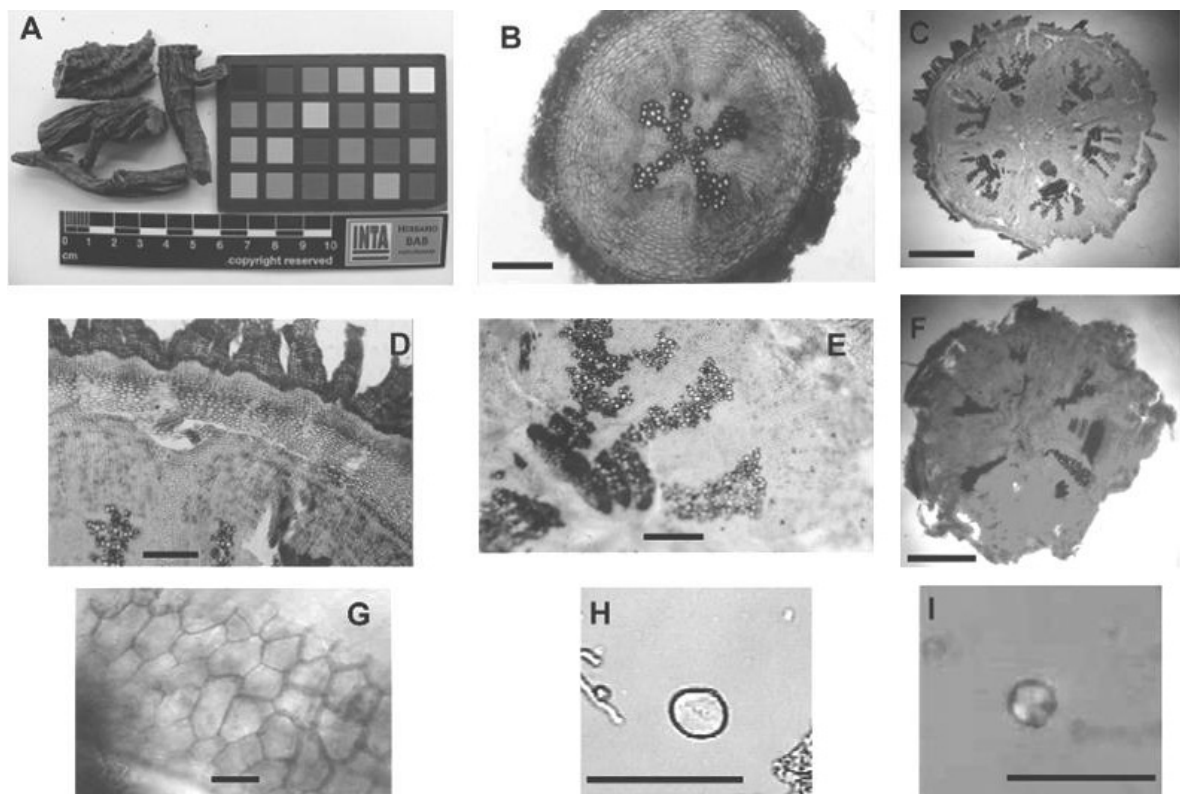
typical structure in tetrarch or pentarch aktinos-tele with early development periderms (Fig. 1 a; Fig. 2 B). In the state of secondary growth, pith was observed (Fig. 2 C). The ratio central cylinder / cortex is low, presenting the cortical parenchyma and the periderm a similar development with five to six layers (Fig. 2 D). The steles presented homogeneous xylem and poorly developed xylem rays (Fig. 1 b; Fig. 2 E).

The cross sections of the rhizome showed a structure with anomalous secondary growth, where xylematic conduction tissue is dispersed in the parenchyma forming irregular groups without phloem and with a fissured disposition. The periderm was found to be poorly developed (Fig. 1 c; Fig. 2 F). Rhizome maceration presented cork with irregular polygonal cells with acute and obtuse angles (Fig. 2 G).

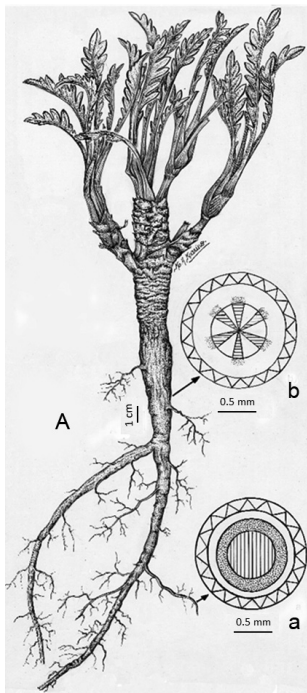
In the powdered subterranean organs, cork cells, tracheas, fibers, and simple, spherical starch grains with a central hilum were observed. The average grains diameter was 10-20 µm, and it showed typical Maltese cross, observed under polarized light (Fig. 2 H, I).



**Figure 1.** *Valeriana carnososa*. **A:** rhizome and roots in external view; **a:** scheme of primary root in cross-section (CS); **b:** scheme of secondary root (CS); **c:** scheme of rhizome (CS).



**Figure 2.** *V. carnososa*. **A:** macroscopic view of rhizomes and roots; **B:** primary root (CS); **C:** secondary root (CS); **D:** secondary root at periderm level (CS); **E:** secondary root at xylem level (CS); **F:** rhizome (CS); **G:** cork in the maceration; **H:** starch grain; **I:** starch grain under polarized light. Bars scales. B, C, D, 2,5 mm; E, F: 354 µm; G, H, I: 50 µm.



**Figure 3.** *Valeriana clarionifolia*. **A.** Roots; **a:** Scheme of primary root (CS); **b:** Scheme of secondary root (CS).

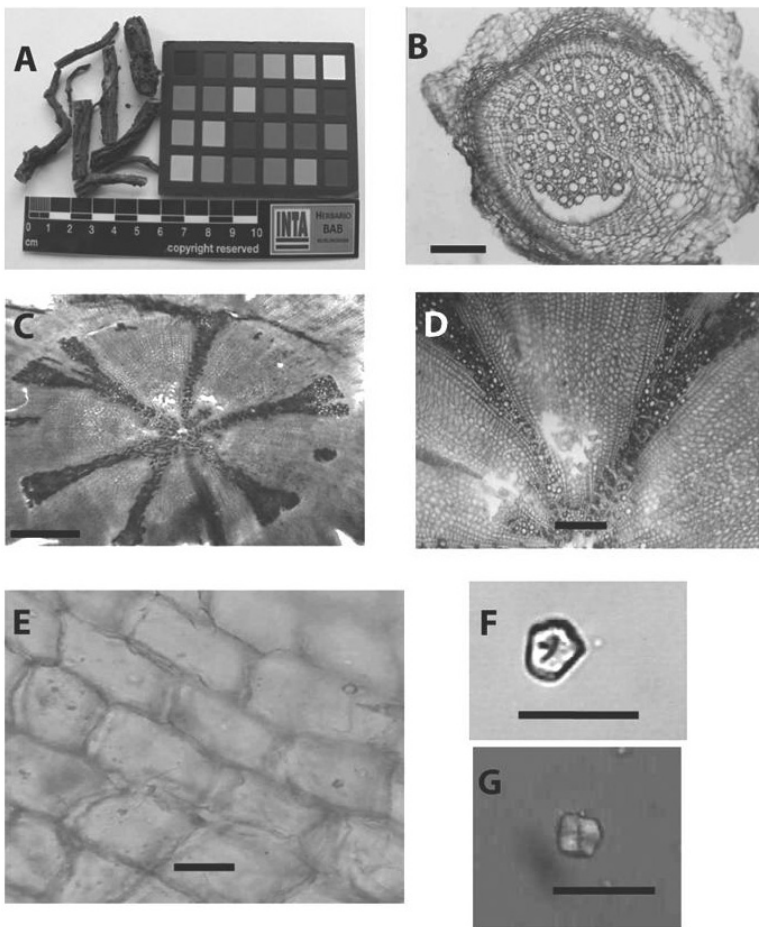
### *Valeriana clarionifolia*

#### Macroscopy

The subterranean system consists of a tap-root up to 4 cm in diameter and may have ramifications. The color is brown, and the surface is rough, without furrowed striations (Fig. 3 A; Fig 4A).

#### Microscopy

Cross section of the root showed an early secondary growth, visible in roots of about 1 mm in diameter. The vascular tissue in the primary structure of the root form an ectofloic proto-stele, therefore no pith is present. Endodermis is not observed. The thickness of the phloem and the parenchyma is variable, with 5-6 cell layers. Cork also present variable thickness with 6-7 cell layers, larger than parenchymal cells (Fig. 3 a; Fig 4 B). In the state of secondary growth, which are the roots used in traditional medicine, the pith is not observed, and the secondary xylem is radially disposed alternating with wide parenchymatic rays. It is surround by secondary phloem. Phloem is difficult to distinguish from parenchyma cells, and the limit



**Figure 4.** *V. clarionifolia*. **A:** macroscopic view of roots; **B:** primary root (CS); **C:** secondary root (CS); **D:** secondary root at central cylinder level (CS); **E:** cork in the maceration; **F:** starch grain; **G:** starch grain under polarized light. Bars scales. B, D: 354  $\mu$ m; C: 1,5 mm; E, F, G: 50  $\mu$ m.

between them is not differentiated to do the absence of phloem fibers. The ratio central cylinder/cortex is 1:1. Periderm is well developed and the ratio cortex / periderm is 2:1 (Fig. 3 b; Fig. 4 C, D).

In the maceration, rectangular cork cells with right angles were observed (Fig. 4 E). Powder examination showed the presence of simple, hexagonal starch grains, with a central hilum, being 15-25  $\mu\text{m}$  in diameter. Observed under polarized light, it showed typical Maltese cross (Fig. 4 F, G).

## DISCUSSION

Among the species of *Valeriana* used in traditional medicine, *V. officinalis* was the first whose subterranean organs anatomy was known. It is characterized by a rhizome from which numerous roots emerge. The rhizome presents a eustele vascular structure, while the primary root has an ectophloic siphonostele<sup>18</sup>. *V. carnososa* is included in the group of species possessing rhizome, but unlike *V. officinalis*, the rhizome of *V. carnososa* presents an anomalous secondary structure.

The anomalous growth occurs in several groups, particularly Dicotyledons, and it is common to refer to them as atypical structures<sup>17</sup> described several types of anomalous growth, but not for Valerianaceae. The xylem arrangement of *Schnella microstachya* Raddi (sub. nom. *Baubinia langsдорфiana* Bong.) (Leguminosae) is similar to that present in *V. carnososa*<sup>17</sup>. In the genus *Valeriana*, Lörcher & Weberling<sup>19</sup> reported a type of anomalous secondary growth in the rhizomes of *V. micropteryna* Wedd and *V. thalictroides* Graebn from Peruvian Andes, where areas of medullar cambium are formed and produce inner xylem which fractures into small portions and may join with each other in ellipsoidal rings. Thus, phloem-free complexes of conducting tissue are formed. This kind of growth with the xylem dispersed throughout the organ and without any order is present in the rhizome of *V. carnososa*.

Moreover, *V. clarionifolia* lacks rhizome and only possesses roots. Due to the exomorphology present in both "ñancolahuen" species, in a trade sample the anomalous structure of *V. carnososa* rhizome should be compared with the secondary roots of *V. clarionifolia*, since these structures represent over 90 % of the subterranean organs of these species.

The macerated material provides a distinctive

diagnostic character. While in *V. clarionifolia* the cork presents quadrangular cells, with right angles, the cork cells of *V. carnososa* are polygonal, with acute and obtuse angles. Also, in *V. officinalis* cork cells are irregular according to Schneider<sup>20</sup>. Therefore, this character can be used to differentiate the two species of "ñancolahuen", and between *V. clarionifolia* and *V. officinalis*.

Starch grains are ergastic substances that can be detected easily without destroying the sample by using polarized light observation; they can also be detected even in powdered plant drugs. It is the case of the two species tested here, which are differentiated by size and morphology of the starch grains: 10-20  $\mu\text{m}$  in diameter and spherical in *V. carnososa*, while in *V. clarionifolia* are 15-25  $\mu\text{m}$  in diameter and hexagonal shape. These two species are different from *V. officinalis* whose starch granules are numerous, highly variable in diameter (3-20  $\mu\text{m}$ ), and are grouped as composite grains<sup>18,20-22</sup>. They can also be differentiated from *V. jatamansi* Jones, because this species has simple or compound granules 10-15  $\mu\text{m}$ , as well as *V. procera* Kunth and *V. edulis* Nutt. These last species have also no starch grains<sup>23</sup>. In native Argentinian species records: *V. ferax* (Griseb) Höck. and *V. polybotrya* (Griseb.) Höck., no starch granules after pharmacognostic studies were found<sup>24</sup>. Since in the Patagonian species starch content is scarce, a thorough examination should be carried out to detect it otherwise it cannot be informed<sup>9</sup>. It is also advisable to analyze more than one population in this type of study.

## CONCLUSION

The morphology and anatomy of subterranean organs of *V. carnososa* and *V. clarionifolia* are described for the first time. The roots of both species can be differentiated through cross-sections. If the material is in very small portions which do not allow making a histological section to see the arrangement of all the tissues, it can be authenticated by observing the cork cells in the maceration and the starch grains in the powdered material.

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