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# Internal secretory structures and chemical compounds in leaves of *Conyza bonariensis* (L.) Cronquist and *Solidago chilensis* Meyen (Asteraceae, Astereae)

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#### ABSTRACT

Conyza bonariensis (L.) Cronquist and Solidago chilensis Meyen (Asteraceae, Astereae) are mainly known as weeds. The aim of this investigation was to characterise their internal secretory structures, establish the main substances accumulated in them, and saponins in the leaves. Plants were collected in Berisso, Gonnet and La Plata, Argentina. Vouchers were deposited in LPAG herbarium. Leaves were conserved in alcohol 70°. Leaves cross sections were obtained, others were made diaphanous, and all were stained with dyes and reagents. Histochemical and phytochemical colorimetric reactions were performed. Cavities in the major veins (1st to 3rd orders) are on phloem side. Above and/ or below vascular bundles occur in the minor veins (4th to 6th orders) and mesophyll in S. chilensis, but cavities are not in veinlets (5th and 6th orders), nor in mesophyll in C. bonariensis. Cavities are surrounded by 1-3 layers of attached parenchyma. In paradermal view, on the major veins the file of cavities is enclosed in a false duct formed by the attached parenchyma cells looking as a true duct. On the minor veins and mesophyll, isolated or small groups of cavities were found. In the spaces of cavities resins, phenolic and lipophilic compounds were identified. Saponins in leaves have been found in both species. The cavities are the internal secretory structures in Conyza bonariensis and Solidago chilensis. They accumulate substances of diverse chemical nature, which would be useful as a resource for medicine and for the biocontrol of pathogens to improve sustainable agricultural systems.

KEY WORDS Asteraceae, cavities, chemical substances, false ducts, reservoirs.

#### RÉSUMÉ

Structures sécrétrices internes et composés chimiques dans les feuilles de Conyza bonariensis (L.) Cronquist et Solidago chilensis Meyen (Asteraceae, Astereae).

Conyza bonariensis (L.) Cronquist et Solidago chilensis Meyen (Astéracées, Astérées) sont principalement connues comme mauvaises herbes. Le but de cette enquête était de caractériser leurs structures sécrétrices internes aux feuilles, et de détecter les principales substances qui y sont accumulées ainsi que les saponines. Les plantes ont été récoltées à Berisso, Gonnet et La Plata, Argentine. Les spécimens d'herbier ont été déposés dans l'herbier du LPAG. Les feuilles conservées dans de l'alcool à 70°, ont été sectionnées transversalement ou on fait l'objet d'unn éclaicissage, toutes ont été traitées par des colorants et des réactifs. Des réactions colorimétriques histochimiques et phytochimiques ont été réalisées. Les cavités des nervures principales (du 1er au 3e ordres) sont du côté du phloème. Elles se retrouvent au-dessus et/ou au-dessous des faisceaux vasculaires dans les nervilles mineures (ordres 4 à 6) et le mésophylle chez S. chilensis, mais sont absentes dans les veinules (ordres 5 et 6), et le mésophylle chez C. bonariensis. Ces cavités sont entourées de 1 à 3 couches de parenchyme attaché. En vue paradermique, sur les veines majeures, la file de cavités est enfermée dans un faux conduit formé par les cellules de parenchyme attachée ressemblant à véritable conduit. Sur les veines mineures et le mésophylle, des cavités isolées ou en petits groupes ont été trouvées. Dans la lumière des cavités, des résines, des composés phénoliques et lipophiles ont été identifiés. Des saponines dans les feuilles ont également été trouvées chez les deux espèces. Ces cavités sont les structures sécrétrices internes de Conyza bonariensis et Solidago chilensis, accumulant des substances de nature chimique diverse, potentiellement utiles à la médecine et au contrôle biologique des pathogènes afin d'améliorer les systèmes agricoles durables.

MOTS CLÉS Asteraceae, cavités, substances chimiques, faux conduits, réservoirs.

#### INTRODUCTION

Conyza bonariensis (L.) Cronquist and Solidago chilensis Meyen belong to the family Asteraceae, subfamily Asteroideae and tribe Astereae. Conyza bonariensis is an annual herb native of South America and adventitious in the rest of the World (Africa, Asia, Central America, Europe, North America and Oceania), frequently transformed into invasive weed. It is an herbicide-resistant weed (Rapoport et al. 2009; Heap 2020; CABI 2021). However, numerous authors have pointed out the medicinal properties of C. bonariensis, e.g., according Alonso & Desmarchelier (2005) the glucosides are antiinflamatory and cough suppressant medicine; the quercetina glucoside also demonstrated antispasmodic and diuretic activities; the essential oils with limonene as major component have anti-inflamatory activity. Barboza et al. (2009) reported that aerial parts have antiulcer, hepatic, antihelmintic properties; the infusion is antipyretic, antirheumatic, diuretic, antiseptic, urinary, antidiarrhoeal, wound healing for acne and gastroprotective. Essential oils have anti-inflamatory, depressant and cardiotonic activities. Manzano Santana et al. (2011) established the anti-inflamatory and antimycotic effect of the alcoholic extract of essential oils from the leaves. Phytochemical screening of C. bonariensis showed the presence of anthocyanidines, anthraquinones, cardiotonic heterosides, cumarins, essential oils, flavonoids, phenolic compounds, quinines, reducing compounds, saponins, sesquiterpenic lactones, tannins, triterpene-steroids (Alonso & Desmarchelier 2005; Hurrell et al. 2011). The mainly essential oils components are sesquiterpenes and minoritary diterpenes (Manzano Santana et al. 2011). Shah et al. (2013) reported that chloroform and ethyl acetate extracts of *C. bonariensis* give the best results for its antibacterial, antifungal and phytotoxic effect.

Solidago chilensis is a perennial herb with ample distribution in North and South America, Europe and Asia (López Laphitz & Semple 2015). This is a toxic plant for livestock and invasive weed (Marzocca 1997). However, S. chilensis is a species adapted to xeric areas which could be used as ornamental and melliferous plant (Gil et al. 2012). Alonso & Desmarchelier (2005) reported that S. chilensis has been used in medicine since colonial times. Several investigations informed medicinal properties and uses of this species, Alonso & Desmarchelier (2005) indicated as antiinflamatory, antioxidant, antiulcerogenic and gastroprotective; Zampini et al. (2007) communicated that the presence of polyphenols would have significant inhibitory activity on antibiotic-resistant bacterias. Bucciarelli et al. (2013) reported that S. chilensis have been used in traditional medicine as anticephalalgic, antiinflamatory, diuretic, and mentioned that the presence of flavonoids, phenolics compounds, carbohydrates, saponins and terpenes would have effect as gastroprotectors. In the last years, the essential oils received attention for their antimicrobial and antioxidant activity (Barboza et al. 2009; Gastaldi et al. 2016). The anatomy of S. chilensis and the presence of essential oils in internal secretory structures were treated by authors (Hernández et al. 2013; De Souza et al. 2018; Pérez et al. 2018; Hernández & Arambarri 2022), and an interesting review was performed by Gastaldi et al. (2018). Frequently, the internal secretory structures are scarcely studied. Lersten & Curtis (1987, 1989) performed the most detailed papers on that. However, it is interesting to know more about the internal secretory structures because they can act transporting



Fig. 1. — Cavities and false duct in cross section view: **A**, Solidago chilensis Meyen, primary vein showing next to phloem a cavity with uniseriate epithelium of eight cells surrounded by one cycled layer of attached parenchyma cells, all enclosed in the parenchyma sheath; **B**, Conyza bonariensis (L.) Cronquist, primary vein showing next to phloem a cavity with uniseriate epithelium of 8 cells surrounded by two cycled layers of attached parenchyma cells, all enclosed in the parenchyma sheath. Abbreviations: **Apc**, attached parenchyma cells; **Cs**, cavity space; **Ec**, epithelial cells; **Ph**, phloem; **Ps**, parenchyma sheath. Scale bars: 100 µm.

substances or as reservoirs of chemical compounds which are involved in the participation of numerous ecological plant relationships (Piñol *et al.* 2008) and on plant defence (Dussourd & Denno 1991). On the basis of the numerous previous investigations, the aims of this research are: 1) to broaden and deepen the knowledge of internal secretory structures types in leaves of *C. bonariensis* and *S. chilensis*; 2) to check the main substances secreted and/or accumulated in the secretory structures; and 3) to establish the qualitative content of saponins in leaves of *Conyza bonariensis* to compare with *Solidago chilensis* in which saponins presence was previously investigated by Arambarri & Hernández (2014).

#### MATERIAL AND METHODS

*Conyza bonariensis* and *Solidago chilensis* plants having fully developed leaves were collected along the roads in the Gonnet, Berisso and La Plata cities, La Plata party (Pdo.), Buenos Aires province (Prov.), Argentina. The botanical material was identified and the vouchers were deposited in the Facultad de Agronomía, Universidad Nacional de La Plata (LPAG) herbarium. Specimens examined are included in the Appendix 1.

For anatomy study, fresh mature leaves from basal, middle and apical stem regions were stored in 70% ethanol. To analyze the structures, freehand cross sections (CS) of leaf blade middle part were cut; the selected sections were bleached in (50%) sodium hypochlorite (NaClO), washed thrice with distilled water (DW), then were stained with an alcoholic solution (80%) safranin or (0.05%) Toluidine blue "O" (O'Brien et al. 1964). For the paradermal view (PW), the leaves were clarified using the method of Franklin (1945) modified, for that, they were boiled in ethyl alcohol 96° for 10 min, allowed to cool then washed and bleached in 50% (NaClO) for 1-2 h, when leaves were slightly discoloured they were removed, washed twice and submerged in a solution of (10 vol.) hydrogen peroxide + (5%) glacial acetic acid (1: 1 v/v) for 48 h, after that washed twice in DW and bleached again in (50%) NaClO. At the completion of the bleaching process, five washes were carried out to remove the NaClO, and samples were transferred into a solution of (5%) chloral hydrate for a minimum of 72 h. To complete the process, leaves were stained using different dyes and reagents, Safranin, Toluidine blue "O", and Oil red "O". The sections and diaphanous leaves were mounted in gelatin-glycerin on glass slides and sealed with nail polish. Cavities dimensions in cross section of 20 samples were taken using ImageJ program (González 2018), they were the width (w) in the direction of the width of the leaf blade, and high (h) in the adaxial-abaxial direction; mean values were expressed in micrometer (µm).

The histochemical analysis was performed on leaves cross sections and paradermal view. To evaluate presence of starch in the parenchyma sheath of the vascular bundles a solution of iodine potassium iodide (IKI) was used (Ruzin 1999), a dark blue colour indicated the starch grains. To analyse lipid compounds an alcoholic solution of Oil red "O" was used (Gurr 1971), the orange to red color indicates positive test and essential oils were seen as droplets. Detection of tannins was performed using (10%) ferric chloride and (2%) sodium carbonate (Zarlavsky 2014), a green-blue color is a positive test. Resins were detected using a saturated solution of copper sulphate and heated gently (Cosa *et al.* 2014) a color emerald green indicates positive test. Toluidine blue "O" was used to contrast phenolic compounds a green turquoise colour indicate positive test (Tapia-Torres *et al.* 2014).

Foam test was used to identified saponins in leaves of *C. bonariensis*, for that the aqueous extract was distributed in ten test tubes (16 mm) filled up with dilutions 1 to 10 mL (extract/DW). The test tubes with solution were longitudinal shaken vigorously 15 s, and after 15 min the persistence of saponins foam was evaluated according the foam column high, a layer of foam measuring about 1cm was formed which indicated positive test (Mac Donald *et al.* 2005; Korwar *et al.* 2010).

Anatomical structures were analysed and microphotographs were taken by using a stereoscopic microscope Bausch & Lomb, stereo zoom 5, and a digital camera, resolution 12 MP; light microscopes Lancet XSP-136D with digital camera solution disk, and a Nikon E200 LED optical microscope equipped with Micrometrics SE Premium software.

For analyse the secretory structures we consider duct (=canal) and cavities according Metcalfe & Chalk (1989) who wrote: "Secretory cavities are intercellular spaces of various sizes and shapes in which chemical substances secreted from the surrounding cells are deposited. Secretory canals differ from cavities in being more elongated, and both are sometimes lined by ephitelial cells from which secretion take place. Duct and cavities are commonly formed by the separation of cells that were previously in contact with each other and when formed in this way they are described as schizogenous". In our understanding the ducts from different plants and families are large intercellular spaces and have continuity with their main function to conduct substances from one place to another, whereas in cavities the intercellular space is shorter given discontinuity and their main function is to store substances acting as reservoirs, named given by Lersten & Curtis (1989).

# RESULTS

# DESCRIPTION OF CAVITIES AND FALSE DUCTS

The leaf blade cross sections exhibited the vascular bundles which often have one secretory structure next to and on the phloem side, all surrounded by the parenchyma sheath. The secretory structure is a shorter intercellular space which has schizogenous origin. This is a cavity, formed by the intercellular space bounded at its periphery by the secretory cells of the uniseriate epithelium which exude substances into the intercellular space or cavity space. In cross section the epithelium appears formed by (4-)5-8(-10) secretory cells. The cavity in cross section is rounded in outline and appears surrounded by one or more layers of parenchyma cells smaller than the rest of parenchyma sheath cells. They are arranged more or less in a cyclical manner enveloping the cavity. We named these parenchyma cells, attached parenchyma cells (Apc). There is



Fig. 2. — Cavities and false duct in paradermal view: **A**, **B**, *Solidago chilensis* Meyen: **A**, false duct in surface, formed by attached parenchyma cells enveloping the cavities; **B**, large cavity showing lipophilic substances in the cavity space, oil droplets in epithelial cells, and attached parenchyma cells; **C**, *Conyza bonariensis* (L.) Cronquist, two cavities with oblique and superimposed ends, surrounded by several layers of attached parenchyma cells. Abbreviations: **Apc**, attached parenchyma cells; **Cd**, oil droplets in epithelial cells; **Ps**, parenchyma sheath; Scale bars: 100 µm.

one layer of Apc in *S. chilensis* and 1-3 layers in *C. bonariensis* (Fig. 1A, B). This attached parenchyma forms a false duct around the cavities looking in a paradermal or longitudinal view as a true duct (Fig. 2A-C). It is the first time that this parenchymal false duct is described.

#### CAVITIES LOCATION AND DIMENSIONS

In *C. bonariensis* and *S. chilensis* the cavities are located on the major veins (1<sup>st</sup>-3<sup>rd</sup> orders) exclusively on abaxial side (near to phloem) (Figs 1A, B; 3A). Sometimes, the primary or secondary vein cross sections do not show a cavity, and the space is occupied by parenchyma cells. It represents the discontinuity of the cavities file (Figs 3B; 4A, B). In *C. bonariensis* the primary and secondary veins never have phloem fibres (Fig. 3A) while they often occur in *S. chilensis* (Fig. 3B). In the 4<sup>th</sup> order veins of both studied species, the cavities appear on the adaxial side, near to xylem, or on both vascular bundle sides (Fig. 3C, D). In the minor veins, in *C. bonariensis* there are no cavities in 5<sup>th</sup> and 6<sup>th</sup> order veinlets, nor in the mesophyll, while in *S. chilensis*, cavities are present in 5<sup>th</sup> and 6<sup>th</sup> order veinlets and in the mesophyll (Figs 4B; 5A).

The measurements of the abaxial cavities varied between 55.7  $\mu$ m width × 52.5  $\mu$ m hight in *Conyza* and 56.6  $\mu$ m w × 49.4  $\mu$ m h in *Solidago* while adaxial cavity dimensions were 66.4  $\mu$ m w × 60.1  $\mu$ m h in *Conyza*, and 63.9  $\mu$ m w × 57.3  $\mu$ m h in *Solidago*. Thus the adaxial cavities resulted larger than the abaxial ones (Fig. 3C, D).

#### CAVITIES AND FALSE DUCT IN PARADERMAL VIEW

In Conyza on the 1st-3rd order veins the cavities are oblong and disposed in a file in contact by their ends or separated by parenchyma cells, and enclosed in the false duct. The quaternary order vein exhibits isolated oblong cavities or small groups of them. The veinlets (5th and 6th order) and the mesophyll do not show cavities (Fig. 4A). In Solidago on the 1st-3rd order veins the cavities are elongated and narrow (linear shape) aligned and enclosed in the false duct (Fig. 4B). On the primary veins the cavities are disposed in a dense file, frequently in contact by their oblique ends (Figs 2A; 5D), frequently separate by only one cell layer which sometimes disappears and the cavities merge together by their ends. Similar to primary vein the cavities are on the secondary veins but a bit separated just like in the tertiary veins, and in quaternary veins the quantity of cavities is being reduced leaving large spaces between them, also the cavities are shorter and oblong (Fig. 4B). Frequently, on the veinlets of 5th and 6th order isolated and sometimes two to three cavities clustered are found (Figs 4B; 5A). Cavity shapes in minor veins are variable, oblong, oblong elongated (Fig. 5A, B), oblong rounded in the mesophyll and at the end of the veinlets (Fig. 5A, C), and when the veins branched out appeared triangular (Fig. 5A, D).

#### CAVITIES AND CHEMICAL COMPOUNDS

The cavity epithelial secretory cells often show droplets of essential oils, sometimes is visible a resinous substance. In the cavity space accumulates a mixture of chemical compounds. In both species using histochemical tests were identified resins, phenolic compounds, lipophilic substances and essential oils. Frequently, in the apical and youngest leaves there are abundant essential oils which give an intense red colour in the epithelial cells when a test for lipophilic substances is performed, and there is an empty space of the cavity. In the mature median leaves the epithelial cells still may content lipophilic substances and the space of the cavity is filled with compounds of different chemical nature. The mature leaves, located in the basal part of stem frequently have the epithelial cells empty and the space of the cavity filled with resins, phenolic compounds, lipophilic substances and oils (Fig. 6A, B, D-F). In the parenchyma sheath were also found starch grains (Fig. 6C).

The saponins in *C. bonariensis* leaves, produced a foam column less than 1 cm in high, thus the saponins content was slightly positive whereas in *S. chilensis* is highly positive because the foam column is more than 2 cm high (Fig. 6G, H).

#### DISCUSSION

#### CAVITIES STRUCTURE AND LOCATION

The internal secretory structures in Conyza bonariensis and Solidago chilensis are cavities disposed in a similar manner. Lersten & Curtis (1987) described them for Conyza canadensis as short secretory channels arranged in a row. According the expression short channels they would be similar to cavities. Lersten & Curtis (1989) studied Solidago canadensis and they named them reservoirs. Conyza bonariensis and S. chilensis showed cavities which would be the reservoirs of Lersten & Curtis (1989) and they already pointed out the existence of the false duct however it is not clear if they attributed it to the parenchyma cell layers surrounding the cavities. In the present study we attribute the false duct to one or more layers of parenchyma cells surrounding the cavities which we refer to as attached parenchyma cells. Our results do not agree with internal secretory structures indicated by Martínez-Quezada et al. (2023) for Asteraceae family, principally because we have different concepts of ducts and cavities, it is evident in their fig. 2d. Another reason could be that the false duct can be confused as a true duct.

The cavities location found is in agreement with those reported for *Conyza canadensis* (Lersten & Curtis 1987); *Solidago canadensis* (Lersten & Curtis, 1989); *Solidago chilensis* (Pérez *et al.* 2018) and *Conyza bonariensis* (Pérez & Apóstolo 2022). In *C. bonariensis* and *S. chilensis* we have also found in some cross sections the primary and secondary veins without cavity. We speculate that this is so because the row of cavities is discontinuous.

### CAVITIES AND CHEMICAL COMPOUNDS

Fahn (2002), stated that the content of secretory tissues has two very important ecological functions: 1) the protective function against herbivores, insects, microbial diseases; and 2) the attractive function for parasitoids and/or pollinators.

The presence of chemical compounds accumulated in the cavities (epithelium and space of the cavity) was found vari-



Fig. 3. — Cavities location in cross section (CS) view: **A**, *Conyza bonariensis* (L.) Cronquist, CS of secondary vein showing vascular bundle without fibres, next to phloem a cavity with lipophilic subtances in epithelial cells surrounded by several layers of attached parenchyma cells (false duct) and parenchyma sheath;; **B-D**, *Solidago chilensis* Meyen: **B**, CS of secondary vein showing fibres on adaxial and abaxial sides, and on phloem side absence of cavity but presence of attached parenchyma cells and parenchyma sheath; **C**, **D**, leaf blade CS showing adaxial and abaxial cavities; **C**, cavities located on different vascular bundles; **D**, cavities on both sides of one vascular bundle. Abbreviations: **ABE**, abaxial epidermis; **ADE**, adaxial epidermis; **Apc**, attached parenchyma cells; **Co**, collenchyma; **Ec**, epithelial cells; **Fb**, fibres; **MES**, mesophyll; **Ph**, phloem; **Ps**, parenchyma sheath; **Xy**, xylem. Scale bars: 100 µm.



Fig. 4. — Cavities (**blue colour**) on abaxial side in paradermal view: **A**, *Conyza bonariensis* (**L**.) Cronquist, showing 1<sup>st</sup> to 3<sup>rd</sup> vein orders with oblong cavities and false duct, and in 4<sup>th</sup> order veins solitary cavity or in small groups; **B**, *Solidago chilensis* Meyen, showing linear cavities enclosed in false duct in the first, second and third vein orders, cavities oblong, triangular, rounded in veins of 4<sup>th</sup> to 6<sup>th</sup> orders. Abbreviations: **1-4**, vein orders. Scale bars: 1 mm.



Fig. 5. — Paradermal view of cavity shapes in *Solidago chilensis* Meyen: **A**, detail of areola, solitary cavities triangular among the veins and round shaped at the end of the minor veins and in the mesophyll; **B**, oblong-elongated cavity; **C**, oblong-rounded cavity at the end of veinlet 6<sup>th</sup> order; **D**, triangular cavity. Abbreviation: **Oe**, oblique end cells. Scale bars: A, 1 mm; B-D, 100 µm.



Fig. 6. – Histochemical and phytochemical tests: A-D, G, *Conyza bonariensis* (L.) Cronquist: A, leaf cross section (CS) showing a cavity with resins (solution of copper sulphate); B, paradermal view (PV) of two cavities, the cavity space filled with resins and phenolic compounds (Toluidine blue "O"); C, starch grains in parenchyma sheath cells (IKI test); D, leaf CS showing droplets in a cavity (Oil red "O"); E, F, H, *Solidago chilensis* Meyen: E, median leaf CS showing two cavities with resins and phenolic compounds; F, leaf CS displaying a cavity with essential oil droplets. Saponins: G, H, foam columns; G, less than 1 cm high; H, more than 2 cm high. Abbreviations: ABE, abaxial epidermis; ADE, adaxial epidermis; MES, mesophyll; Od, oil droplets; Pc, phenolic compounds; Rs, resins; Sg, starch grains. Scale bars: A-F, 100 µm; G, H, 1 cm.

able with the age of leaves and with their level in the stem. The observation on major content of essential oils in juvenile leaves would be in agreement with Kinupp (2007) who reported on *C. bonariensis*, that the young leaves (before the plant flowers) are aromatic and spicy, used as a seasoning for meats or added to salads. The mature leaves, located in the basal part of stem frequently have the cavity space filled with resins, phenolic compounds, lipophilic substances. In these basal leaves the cavities act as reservoirs, and when leaves are cut or during decomposition in the ground release the chemical compounds which have an ecological role (e.g., allelopathic effects that inhibit seed germination or growth of other plants) (Langenheim 1994).

The resins are a complex of substances mainly nonvolatile terpenoids which reduced palatability. Schuck (2007) suggested that resins, such as monoterpenes, may act toxically, whereas another fraction, such as diterpenes, may act as a physical barrier in defending against pathogenic fungi. Resins have the first protective function of Fahn (2002).

The phenolic compounds (phenols, flavonoids, tannins) according Castro & Demarco (2008) have protective function. They play a role in defence against pathogens and are deterrent to herbivores by reducing the digestibility of nutrients, and protect plants against damage by UV radiation.

The essential oils are volatile terpenoids. The presence of these volatile organic compounds is of great interest because in addition to the medicinal properties, they are elements of plant chemical communication due to their volatility and aroma, to attract pollinators or seed dispersers, as a defence stopping antimicrobial activity (bactericidal, fungicidal) (Marín-Loaiza & Céspedes 2007).

The saponins are present in leaves of *C. bonariensis* and *S. chilensis*. The saponins have medicinal applications. On the other hand, they are toxic for insects, parasite worms (an-thelmintic activity), mollusk and fish; and act as antifungal, antibacterial, antiviral agents (Francis *et al.* 2002; Lanzotti *et al.* 2012). Therefore, saponins are useful to develop new biocontrol agents compatible with a sustainable agriculture.

# CONCLUSIONS

*Conyza bonariensis* and *Solidago chilensis* have cavities as internal secretory structures, located predominantly on abaxial side of vascular bundles and enclosed in a false duct.

In both species the main chemical compounds identified in cavities were resins, phenolic compounds (flavonoids, anthocianidins, tannins) and lipophilic substances (essential oils).

Saponins are present in both species however the foam column is notably higher in *Solidago* than in *Conyza*.

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# REFERENCES

- ALONSO J. & DESMARCHELIER C. 2005. Plantas medicinales autóctonas de la Argentina: bases científicas para su aplicación en la atención primaria de la salud. Corpus libros Médicos y Científicos, Buenos Aires, Argentina, 664 p.
- ARAMBARRI A. & HERNÁNDEZ M. P. 2014. Variación estacional de saponinas en Solidago chilensis var. chilensis (Asteraceae). Boletín de la Sociedad Argentina de Botánica 49: 483-489. https:// doi.org/10.31055/1851.2372.v49.n4.9882
- BARBOZA G., CANTERO J., NÚÑEZ C., PACCIARONI A. & ARIZA ESPINAR L. 2009. — Medicinal plants: A general review and a phytochemical and ethnopharmacological screening of the native Argentine Flora. *Kurtziana* 34: 7-365. http://hdl.handle. net/11336/24328
- BUCCIARELLI A., ORESTI G., MORENO M., SKLIAR M. & AVELDAÑO M. 2013. — Composición de ácidos grasos de inflorescencias de *Solidago chilensis* (Asteraceae), especie nativa de interés medicinal. *Dominguezia* 29: 56-57. http://hdl.handle.net/11336/233647
- CABI 2021. Conyza bonariensis. En invasive species compendium. Walllingford, UK: Cab International. https://doi.org/10.1079/ cabicompendium.15250
- CASTRO M. DE M. & DEMARCO D. 2008. Phenolic compounds produced by secretory structures in plants: a brief review. *Natural Product Communications* 3: 1273-1284. https://doi. org/10.1177/1934578X0800300809
- COSA M., DOTTORI N., STIEFKENS L., HADID M., MATESEVACH M., DELBÓN N., WIEMER P., MACHADO S., CABRERA V., COSTA C., PÉREZ A. & TRENCHI A. 2014. — Aplicación de técnicas de histología vegetal a la resolución de diversos problemas. Laboratorio de Morfología Vegetal, Universidad Nacional de Córdoba, Argentina, 133 p.
- DE SOUZA D., SA R., ARAÚJO E. & RANDAU K. 2018. Anatomical, phytochemical and histochemical study of *Solidago chilensis* Meyen. *Anais da Academia Brasileira de Ciências* 90: 2107-2120. https://doi.org/10.1590/0001-3765201720160280
- DUSSOURD D. & DENNO R. 1991. Deactivation of plant defense: correspondence between insect behavior and secretory canal architecture. *Ecology* 72: 1383-1396. https://doi.org/10.2307/1941110
- FAHN A. 2002. Functions and location of secretory tissues in plants and their possible evolutionary trends. *Israel Journal of Plant Sci*ences 50: 59-64. https://doi.org/10.1560/Ljut-M857-Tcb6-3fx5
- FRANCIS G., KEREM Z., MAKKAR H. & BECKER K. 2002. The biological action of saponins in animal systems: a review. *British Journal* of Nutrition 88: 587-605. https://doi.org/10.1079/Bjn2002725
- FRANKLIN G. 1945. Preparation of thin sections of synthetic resins and wood-resin composites, and a new macerating method for wood. *Nature* 155: 51. https://doi.org/10.1038/155051a0
- GASTALDI B., ASSEF Y., VAN BAREN C., DI LEO LIRA P., RETTA D., BANDONI A. & GONZÁLEZ S. 2016. — Actividad antioxidante en infusiones, tinturas y aceites esenciales de especies nativas de la Patagonia Argentina. *Revista Cubana de Plantas Medicinales* 21: 51-62 [retrieved from https://revplantasmedicinales.sld.cu/ index.php/pla/article/view/306]
- GASTALDI B., CATALÁN C., SILVA-SOFRÁS F. & GONZÁLEZ S. 2018. — Solidago chilensis Meyen (Asteraceae), a medicinal plant from South America. A comprehensive review: ethnomedicinal uses, phytochemistry and bioactivity. Boletin Latinoamericano y del Caribe de plantas medicinales y aromáticas 17: 17-29. http:// hdl.handle.net/11336/119950
- GIL S., SEISDEDOS L., REYNA M. & CERANA M. 2012. Epidermis foliar de tres especies de Asteráceas nativas de Argentina con potencial ornamental. *International Journal of Experimental Botany*, Phyton 81: 205-210.
- GONZALEZ A. M. 2018. Image J: una herramienta indispensable para medir el mundo biológico. Sociedad Argentina de Botánica. *Folium* 1: 6-17 (translated to Spanish). http://hdl. handle.net/11336/82733

- GURR E. 1971. Synthetic Dyes in Biology, Medicine and Chemistry. Academic Press, London, 814 p.
- HEAP I. 2020. The International Survey of Herbicide-Resistant Weed Database. https://www.weedscience.org (accessed: 6th Jan 2023).
- HERNÁNDEZ M. P., MARTÍNEZ ALONSO S., MORANDI L. & ARAM-BARRI A. 2013. — Anatomical and chemical analysis in *Solidago* chilensis var. chilensis (Asteraceae). Latin American Journal of Pharmacy 32: 1236-1240.
- HERNÁNDEZ M. P. & ARAMBARRI A. 2022. Estructuras de secreción interna y externa de aceites esenciales en Solidago chilensis (Asteraceae), su distribución en los órganos de la planta. Innova Biology Sciences 2: 15-23. https://doi.org/10.58720/ibs.v2i2.39
- HURRELL J., ULIBARRI E., ARENAS P. & POCHETTINO M. 2011. Plantas de herboristería, Literature of Latin America, Buenos Aires, Argentina, 242 p.
- INSTITUTO DE BOTÁNICA DARWINION 2023 (continuously updated). — http://www2.darwin.edu.ar/floraargentina//fa.htm (accessed: 6th Jan 2023)
- KINUPP V. 2007. Plantas alimentícias não-convencionais da região metropolitana de Porto Alegre, Rs. Repositorio digital Lume, Facultad Agronomía, Universidad Federal de Río Grande do Sul, Brasil, 562 p. https://www.lume.ufrgs.br/handle/10183/12870
- Korwar P., Beknal A., Patil B., Halkai M., Kulkarni U., HARIPRASANNA R. & SOODAM S. 2010. — A study on phytochemical investigation of Drynaria quercifolia Linn. rhizome. International Journal of Pharmaceutical Sciences and Research 1: 148-158. https://doi.org/10.13040/IJPSR.0975-8232.1(12).148-58
- LANGENHEIM J. H. 1994. Higher plant terpenoids: a phytocentric overview of their ecological roles. Journal of Chemical Ecology 20: 1223-1280. https://doi.org/10.1007/Bf02059809
- LANZOTTI V., BARILE E., ANTIGNANI V., BONANOMI G. & SCALA F. 2012. — Antifungal saponins from bulbs of garlic, Allium sativum L. var. Voghiera. Phytochemistry 78: 126-134. https:// doi.org/10.1016/j.phytochem.2012.03.009
- LERSTEN N. R. & CURTIS J. D. 1987. Internal secretory spaces in Asteraceae. A review and original observations on Conyza canadensis (Tribe Astereae). La Cellule 74: 179-196.
- LERSTEN N. R. & CURTIS J. D. 1989. Foliar oil reservoir anatomy and distribution in Solidago canadensis (Asteraceae, tribe Astereae). Nordic Journal of Botany 9: 281-287. https://doi. org/10.1111/j.1756-1051.1989.tb01003.x
- LOPEZ LAPHITZ R. & SEMPLE J. 2015. A multivariate morphometric analysis of the Solidago chilensis group in South America and related taxa in North America (Asteraceae, Astereae). Annals of the Missouri Botanical Garden 100: 423-441. https://doi. org/10.3417/201402
- MAC DONALD D., VALENCIA E., CUYOS M. & DUEÑAS R. 2005. Extracción, identificación y evaluación de saponinas en Agaricus bisporus. Biotempo 5: 31-36. https://doi.org/10.31381/biotempo.v510.889
- MANZANO SANTANA P., MIRANDA M., GUTIÉRREZ Y., GARCÍA G., ORELLANA T. & ORELLANA-MANZANO A. 2011. — Efecto antiinflamatorio y antimicótico del extracto alcohólico y composición química del aceite de hojas de Conyza bonariensis (L.) Cronquist (canilla de venado). Revista Cubana de Plantas Medicinales 16: 13-23. http://scielo.sld.cu/scielo.php?script=sci\_abstract&pid =S1028-47962011000100002 (accessed: 7th Jan 2023)

- MARÍN-LOAIZA J. & CÉSPEDES C. 2007. Compuestos volátiles de plantas, origen, emisión, efectos, análisis y aplicaciones al agro. Revista Fitotecnia Mexicana 30: 327-351.
- MARZOCCA A. 1997. Vademécum de malezas medicinales de la Argentina: indígenas y exóticas. Orientación Gráfica Editora, Buenos Aires, Argentina, 363 p.
- MARTÍNEZ-QUEZADA D. M., RIVERA P., ROJAS-LEAL A., VILLASEÑOR J. & TERRAZAS T. 2023. - Leaf secretory structures in Asteraceae: a synthesis of their diversity and evolution. The Botanical *Review* 89: 59-90. https://doi.org/10.1007/s12229-022-09276-4 METCALFE C. R. & CHALK L. 1989. — *Anatomy of Dicotyledons*.
- 2<sup>nd</sup> Ed., Vol. 2. Clarendon Press, Oxford: 64-69.
- O'BRIEN T., FEDER N. & MCCULLY M. 1964. Polychromatic stained of plant cell walls by toluidine blue O. Protoplasma 59: 368-373. https://doi.org/10.1007/Bf01248568
- PÉREZ B. & APÓSTOLO N. 2022. Morfoanatomía comparada del vástago adulto de Conyza bonariensis var. bonariensis y C. bonariensis var. angustifolia (Asteraceae). Bonplandia 31: 27-46. https://doi.org/10.14482/INDES.30.1.303.661
- PÉREZ B., CARRETTONI D., LUS B. & APÓSTOLO N. 2018. Anatomía del rizoma y de la parte basal, media y distal del vástago de Solidago chilensis var. chilensis (Asteraceae). Lilloa 55: 49-66. https://doi.org/10.30550/j.lil/2018.55.1/5
- PIÑOL M., PALAZÓN J. & CUSIDÓ R. 2008. Introducción al metabolismo secundario, in AZCÓN-BIETO J. & TALÓN M. (eds), Fundamentos de Fisiología Vegetal, 2nd ed. McGraw-Hill Interamericana, Madrid: 323-348.
- RAPOPORT E., MARZOCCA A. & DRAUSAL B. 2009. Malezas comestibles del Cono Sur y otras partes del planeta. Instituto Nacional de Tecnología Agropecuaria (Inta), Buenos Aires, Argentina, 215 p.
- RUZIN S. E. 1999. - Plant Microtechnique and Microscopy. University Press, Oxford, 323 p.
- SHAH N., MUHAMMAD N., AZEEM S., KHAN A., SAMIE M. & KHAN H. 2013. — Antimicrobial and phytotoxic properties of Conyza bonariensis. Pharmacy and Pharmacology Research 1: 8-11 [retrieved from https://www.netjournals.org/z\_PPR\_13\_015.html].
- SCHUCK H. J. 2007. The chemical composition of the monoterpene fraction in wounded wood of Picea abies and its significance for the resistance against wood infecting fungi. European Journal of Forest *Pathology* 12: 175-181. https://doi.org/10.1111/j.1439-0329.1982. tb01391.x
- TAPIA-TORRES N., PÉREZ-OLVERA C., ROMÁN GUERRERO A., QUINTANAR-ISAÍAS A., GARCÍA-MÁRQUEZ E. & CRUZ-SOSA F. 2014. — Histoquímica, contenido de fenoles totales y actividad antioxidante de hoja y de madera de Litsea glaucescens Kunth (Lauraceae). Madera y Bosques 20: 125-137 [retrieved from https:// www.redalyc.org/articulo.oa?id=61732732011].
- ZAMPINI I., CUDMANI N. & ISLA M. 2007. Actividad antimicrobiana de plantas medicinales argentinas sobre bacterias antibiótico-resistentes. Acta Bioquímica Clínica Latinoamericana 41: 385-393 [retrieved from http://www.redalyc.org/articulo. oa?id=53541313].
- ZARLAVSKY G. E. (ed.) 2014. Histología vegetal: Técnicas simples y complejas. Sociedad Argentina de Botánica, Buenos Aires, Argentina: 198 p.

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#### APPENDIX 1. - Studied material.

#### Argentina

Conyza bonariensis

**Prov. Buenos Aires**: Pdo. La Plata, La Plata (34°55'25"S, 57°56'26"W), 23.XI.2022, *Arambarri 530* (LPAG); Pdo. La Plata, Gonnet (34°53'12"S, 58°01'34"W), 12.XI.2022, *Hernández MP 214* (LPAG); 12.XII.2022, *Hernández MP 216* (LPAG). **Prov. Buenos Aires**: Pdo. San Nicolás de los Arroyos, San Nicolás, 6.III.1941, *A. L. Cabrera 7169* (SI) (image 20); Pdo. La Plata, La Plata Bosque, 28.II.1961, *A. L. Cabrera 7174* (SI) (image 19).

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Prov. Buenos Aires: Pdo. Berisso (34°50'40"S, 57°50'48"W), XI.2014, *Hernández MP 99-103* (LPAG); Pdo. La Plata, Gonnet, 20.X.2022, *Hernández MP 211* (LPAG); 15.XI.2022, *Hernández MP 212, 213* (LPAG); 7.XII.2022, *Hernández MP 215* (LPAG). In addition, specimens from Instituto de Botánica Darwinion (SI) were consulted.

Prov. Buenos Aires: Pdo. Guaminí, Guaminí, médanos fijos, 22.III.1938, A. L. Cabrera 4351 (SI) (image 6); Pdo. General Lavalle, San Clemente, 31.I.1939, A. L. Cabrera 4954 (SI) (image 4).