Vol.66(spe): e23230070, 2023
https://doi.org/10.1590/1678-4324-ssbfar-2023230070
ISSN 1678-4324 Online Edition
II South-Brazilian Symposium on Pharmacognosy

Article - Health Science/Pharmacognosy

Trichome Microscopy as a Diagnostic Tool for Species of the *Solidago* Complex: *S. chilensis* and *S. microglossa*

Irailson Thierry Monchak[1]
https://orcid.org/0000-0002-9522-1433

Marí Castro Santos[2]
https://orcid.org/0000-0002-3606-7085

Mariana Koetz[2]
https://orcid.org/0000-0002-9101-5560

Vijayasankar Raman[3]
https://orcid.org/0000-0001-7368-9644

Miriam Anders Apel[4]
https://orcid.org/0000-0003-1288-2293

Amelia Teresinha Henriques[2]
https://orcid.org/0000-0002-1928-3771

Jane Manfron[1,3,5*]
https://orcid.org/0000-0003-1873-2253

[1]Universidade Estadual de Ponta Grossa, Departamento de Ciências Farmacêuticas, Ponta Grossa, Paraná, Brasil; [2]Universidade Federal do Rio Grande do Sul, Faculdade de Farmácia, Porto Alegre, Rio Grande do Sul, Brasil. [3]University of Mississippi, National Center for Natural Products Research, Oxford, Mississippi, United States; [4]Universidade Federal do Rio Grande do Sul, Programa de Pós-graduação em Ciências Farmacêuticas, Porto Alegre, Rio Grande do Sul, Brasil; [5]Universidade Estadual de Ponta Grossa, Programa de Pós-graduação em Ciências Farmacêuticas, Ponta Grossa, Paraná, Brasil.

Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Paulo Vitor Farago

Received: 21-Jan-2023; Accepted: 26-Jul-2023

*Correspondence: jane@uepg.br (J. M.)

HIGHLIGHTS

- *Solidago* comprises two very similar South American species: *S. chilensis* and *S. microglossa*.
- *S. chilensis* and *S. microglossa* can be differentiated microscopically.
- Trichomes are sufficient to differentiate *S. chilensis* and *S. microglossa* even in a powder form.
- The absence or presence of the uniseriate and/or biseriate no glandular trichomes determines the species.

Abstract: The plant species identification process is one of the most critical stages of quality control of plant raw material. Microscopy serves as the most accessible means for this purpose. Epidermal appendages, such as trichomes, are important botanical markers of plant drugs, taking into account their presence or absence and micromorphology. Trichomes are useful in detecting adulterants or substitutes and in authenticating plant raw materials. The genus *Solidago* includes two morphologically similar South American species, *S. chilensis* Meyen and *S. microglossa* DC. (syn. *S. chilensis* var. *megapotamica*). The two species belong to the "*S. chilensis*" complex and are commonly called arnica-do-mato or arnica. They are

morphologically separated primarily on the basis of the average length of the non-glandular trichomes present in the stems. The objective of this study was to search for microscopic markers for the identification and differentiation of these two species to support species identification and quality control of the vegetable raw material. For this purpose, the usual techniques of light and scanning electron microscopy were used. Five types of trichomes were found in this study: I. Simple non-glandular trichome, II. Uniseriate non-glandular trichome, III. Biseriate non-glandular trichome, IV. Flagelliform glandular trichome, and V. Capitate glandular trichome. *S. chilensis* has simple non-glandular, flagelliform glandular, and rarely capitate glandular trichomes. Whereas, in addition to its glandular trichomes, *S. microglossa* has two types of non-glandular trichomes: biseriate and uniseriate. Thus, the two South American species of *Solidago* can be microscopically identified and differentiated based on the presence and absence of the characteristic trichomes. These differences can serve as pharmacognostic subsidies for quality control of the evaluated species.

Keywords: anatomy; arnica; arnica-do-mato; microscopy; quality control.

INTRODUCTION

Quality control of herbal drugs must be carried out at all stages of producing herbal medicines. Microscopy is an efficient and inexpensive method widely used in the identification and authentication of botanicals. Since medicinal plants are primarily marketed in fragmented or powdered form, microscopic characteristics are among the first parameters for quality control [1]. Particularly, microscopic structures are valuable for analyzing plant materials whose features are often not radically changed from fresh material. The drying and fragmentation of the plants do not result in the loss of the most relevant microscopic characteristics. Therefore, the microscopic features are the most stable of the plant when it comes to authentication and detection of adulterants and substitutes [1–3].

Trichomes are important botanical markers in systematic investigations, identification and diagnosis of fragmented and powdered plant drugs. Several studies have demonstrated that the micromorphology of trichomes is diagnostic at the family, genus or even species level and that they can aid in the detection of adulterants or substitutes of vegetable raw materials, even when the sample is pulverized [1, 4, 5, 6].

Species of different genera, for example, *Baccharis*, *Mikania*, *Schinus* and *Solidago*, are often misidentified due to their nonspecific common names and similar morphologies [1, 7, 8]. In the case of *Solidago*, the genus includes two closely similar South American species, *S. chilensis* Meyen and *S. microglossa* DC. (syn. *S. chilensis* var. *megapotamica*). Within the *S. chilensis* complex, the two *Solidago* species exhibit similar morphological characteristics and share the same names, "arnica-do-mato" and "arnica" [9]. Laphitz & Semple (2015) distinguished the two species mainly based on the average length of the non-glandular trichomes on the stem: *S. chilensis* with short trichomes (0.1-0.4 mm; mean of 0.2 ± 0.1 mm long), and *S. microglossa* with longer trichomes (0.4-1.2 mm; mean of 0.9 ± 0.15 mm long) [9].

Considering the confusion in identifying *S. chilensis* and *S. microglossa* due to their similar local names and morphology, this study aimed to analyze the samples collected from different geographical locations to determine microscopic markers for differentiation of these species and quality control of botanical materials traded as "arnica-do-mato".

MATERIAL AND METHODS

Solidago samples were collected from 15 locations in southern Brazil (Table 1). The voucher specimens were identified and deposited in the Herbarium of the Institute of Natural Sciences (ICN), Brazil. Access to the botanical material was approved and licensed by CGEN/SISGEN and registered under code A39B223.

Table 1. Collection information of the *Solidago* samples used in this study

Sample	Species	Herbarium ID	Collection location	Elevation (ASL)	GPS Coordinates
1	*S. chilensis*	ICN 205505	Rosário do Sul, RS	102 m	30º 17’ 53” S, 54º 59' 23" W
2	*S. chilensis*	ICNI 205504	Porto Alegre, RS	58 m	30º 01’51” S 51º 08' 02" W
3	*S. microglossa*	HUCS 16898	Ponta Grossa, PR	975 m	25º 14’ 17” S 50º 0’ 39” W

Cont. Table 1

4	*S. chilensis*	ICN 205506	Sapucaia do Sul/RS	38 m	29º 48’ 54” S 51º 08’ 20” W
5	*S. chilensis*	ICN 205507	Farroupilha/RS	728 m	29º 14’16” W 51º 20’ 06” W
6	*S. microglossa*	ICN 205511	Charqueada/RS	30 m	29º 57’46” S 51º 37’45” W
7	*S. chilensis*	ICN 205508	Caxias do Sul/RS (1)	862 m	29º 07’ 55” S 51º 07’ 12” W
8	*S. chilensis*	HUCS 53334	Caxias do Sul/RS (2)	798 m	29º 07’ 56” S 51º 07’ 11” W
9	*S. microglossa*	ICN 205511	Bom Princípio/RS	20 m	29º 30’ 07” S 51º 21’ 14” W
10	*S. microglossa*	MO 3378118	Caxias do Sul/RS (3)	862 m	29º 07’ 55” S 51º 07’ 12” W
11	*S. chilensis*	ICN 205509	São Leopoldo/RS	55 m	29º 48’ 18” S 51º 08’ 06” W
12	*S. chilensis*	ICN 205510	Guaíba/RS	16 m	30º 03’ 55” S 51º 25’ 43” W
13	*S. microglossa*	MBM 191012	Palmeira/PR	865 m	25º 25’ 28” S 50º 0’ 17” W
14	*S. microglossa*	ICN 205511	União da Serra/RS	520 m	28º 47’ 36” S 52º 2’ 3” W
15	*S. microglossa*	ICN 205512	Garopaba/SC	8 m	28º 01’ 30” S 48º 37’ 19” W

All the *Solidago* specimens were first identified using the stem trichome length criterion described by Laphitz & Semple [9]. Leaf and stem samples of each collection were fixed in FAA 70 [10] and stored in a 70% (*v/v*) ethanol solution [11]. Transverse sections of the samples were made freehand using razors, double staining with astra blue and basic fuchsin, and semi-permanent slides were prepared [12]. For epidermal studies, leaf tissues were clarified in sodium hypochlorite solution for 24-48 h and washed in distilled water. The tissues were treated in a 5% acetic acid solution to neutralize the pH, rewashed in distilled water, and stained in 1% safranin [13]. The slides were mounted with 50% (*v/v*) glycerin [10]. Microscopic analyses were performed using an Olympus CX 31 photonic microscope, and photomicrographs were prepared using a C7070 digital camera attached to the microscope.

For scanning electron microscopy (SEM), the FAA-fixed samples were dehydrated in an increasing ethanolic series and dried in a Balzers CPD-010 critical point dryer. The samples were mounted on aluminum stubs using glued carbon tabs and coated with gold in an IC-50 Ion Coater metallizer (SHIMADZU, Kyoto, Japan). Then, the samples were observed in a Mira 3 field emission SEM (TESCAN, Brno, Czech Republic) and imaged using the Electron Optical Design application.

Both the images obtained from SEM and those from optical microscopy were compared, reviewed and discussed in order to confirm the morphological characteristics observed in each microscopic technique.

RESULTS AND DISCUSSION

The presence of several types of glandular and non-glandular trichomes is a common feature in Asteraceae family [14-16, 17] and *Solidago* genus [18]. In this study, five types of trichomes were found using microscopy methods: I. Simple non-glandular trichome (Figure 1 a, b, c), formed by 5–13 cells, the base can be uniseriate, biseriate or multiseriate, with the last cells elongated and the apical cell tapered with evident rough cuticle. The presence of styloid-type crystalline sand was observed in the first 3 cells at the base of this trichome.

The length of this trichome was used by Laphitz & Semple (2015) as a criterion for differentiating between *S. chilensis* and *S. microglossa*. However, Berhin and coauthors [19] stated that although the length is characteristic can help differentiate such aspects, it can not be used to do so, since trichomes can develop, grow, and become dense based on the plant's response to biotic and abiotic conditions, resulting in structures of different sizes and dimensions.

Simple non-glandular trichomes as observed in the present work have been reported for several genera of Asteraceae family, such as *Baccharis* [1, 4, 15], *Calea* [14] and *Solidago* [18]. The presence of crystals in trichomes is an additional characteristic helpful in species identification [20]. The occurrence of crystals in trichomes was previously mentioned in some members of the family Asteraceae including *Baccharis punctulata* DC. and *B. sphenophylla* Dusén ex Malme [4], *Helianthus annuus* L., *H. tuberosus* L. [21] and *Sigesbeckia jorullensis* Kunth [22].

Trichome type II is uniseriate non-glandular (Figure 1 i), formed by 4–12 cells and measuring about 25 µm in length. Some of them can present base biseriate. Uniseriate non-glandular trichomes were also found in Asteraceae family, such as in *Mikania* genus [23]. Type III is biseriate non-glandular trichome (Figure 1 g, h), formed by 2 series with a variable number of cells (4–12) and measuring between 40 and 160 µm in length.

Type IV, flagelliform glandular trichome (Figure 1 d, e), formed by 1–3 cells at the base (60 µm on average) and an apical cell from which an elongated cylindrical tube, translucent, containing essential oil. This trichome is frequently found in species of the family Asteraceae [4, 16, 24, 25]. Type V, capitate glandular trichome (Figure 1 d, f), consists of a uniseriate or biseriate pedicel of 1–6 cells measuring 30–50 µm, and a delicate, rounded to claviform head formed by 2-3 cells.

Of the fifteen samples analyzed, eight corresponded to *S. chilensis* and seven to *S. microglossa*. The samples of *S. chilensis* showed simple non-glandular trichomes (type I) measuring 0.2-0.6 mm. In comparison, samples of *S. microglossa* showed 0.4-1.1 mm long simple non-glandular trichomes. In addition to the Type I trichomes, both species showed flagelliform glandular (type IV) and rare capitate glandular trichomes (type V). However, uniseriate non-glandular trichomes (type II) and biseriate non-glandular (type III) trichomes were only observed in the samples of *S. microglossa*. The trichomes found were used to compose Table 2, in order to simplify the understanding of the study.

These characteristics can serve as additional markers for species identification and authentication of the *S. chilensis* complex, in addition to providing informative characters for the reconstruction of the evolution of the genus *Solidago.*

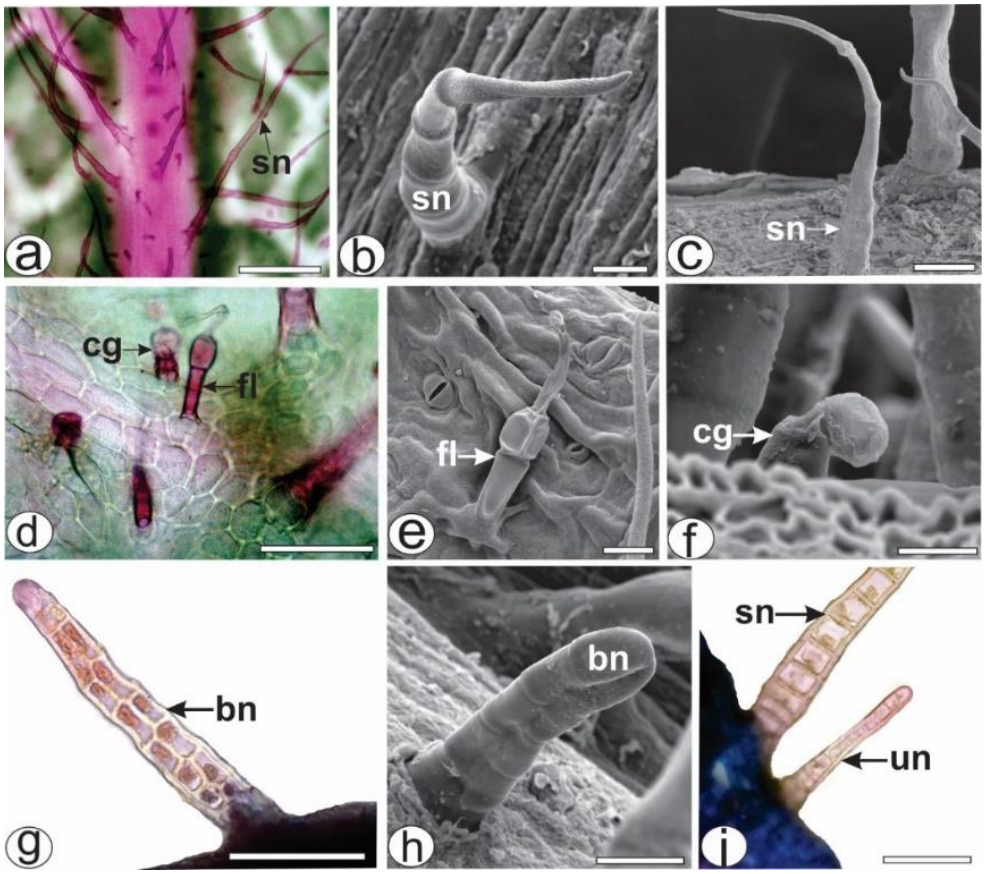

Figure 1. SEM images of the trichomes found in *Solidago* species. a-c, i: Simple non-glandular; d, e: Flagelliform glandular; d, f: capitate glandular; g, h: biseriate non-glandular and i: uniseriate non-glandular. (a-f - *S. chilensis*; g-l - *S. microglossa*). [bn – biseriate non-glandular; cg – capitate glandular; fl – flagelliform glandular; sn – simple non-glandular; un – uniseriate non-glandular]. Scale bars: c = 500 µm; a = 300 µm; d, g, I = 50 µm; b, e, f, h = 20 µm.

Table 2. Trichome types found in *Solidago chilensis* and *S. microglossa*

	Types of trichomes				
***Solidago* species (leaf and stem)**	Simple non-glandular (type I)	Uniseriate non-glandular (type II)	Biseriate non-glandular (type III)	Capitate glandular (type IV)	Flagelliform glandular (type V)
S. chilensis	Present (0.2-0.6 mm long)	Absent	Absent	Present	Present
S. microglossa	Present (0.4-1.1 mm long)	Present	Present	Present	Present

CONCLUSION

Regardless of whether the samples are fragmented or powdered, *S. chilensis* and *S. microglossa* can be differentiated microscopically, using only the set of trichomes present in stems and leaves.

One unique difference between the species is the lack of uniseriate non-glandular and biseriate non-glandular trichomes in species of *S. chilensis*, while the other trichomes – simple non-glandular, capitate glandular, and flagelliform are common to both species of the present study.

Acknowledgments: CAPES (Finance Code 001), Cnpq, C-LABMU, UEPG and Fundação Araucária (Finance Code BIC/FA – 52/2021).
Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Manfron J. Farmacobotânica: uma ferramenta importante para a detecção de adulterações em matérias-primas vegetais. In: Baratto LC, editor. A Farmacognosia no Brasi: memórias da Sociedade Brasileira de Farmacognosia. Petropolis, RJ: Sociedade Brasileira de Farmacognosia; 2021. p. 259-76.
2. Pauzer MS, Borsato TO, Almeida VP, Raman V, Justus B, Pereira CB, et al. *Eucalyptus cinerea*: Microscopic Profile, Chemical Composition of Essential Oil and its Antioxidant, Microbiological and Cytotoxic Activities. Braz Arch Biol Technol. 2021; 64(spe).
3. Machado CD, Santos VLP, Novak RS, Koch MS, Arcaro G, Raman V, et al. Contributions of trichome micromorphology to the characterization of species traded as “BOLDO.” Flora. 2021 Jun; 279(3):151827.
4. Budel JM, Raman V, Monteiro LM, Almeida VP, Bobek VB, Heiden G, et al. Foliar anatomy and microscopy of six Brazilian species of *Baccharis* (Asteraceae). Microsc Res Techniq. 2018 Aug; 81(8):832–42.
5. Antunes MN, Pereira FR, Leitão CAE. Structural Characterisation of the Leaf of *Bauhinia monandra* Kurz (Fabaceae – Cercidoideae). Braz Arch Biol Technol. 2021; 64.
6. Justus B, Almeida VP, Gonçalves MM, Assunção DPSF, Borsato DM, Arana AFM, et al. Chemical composition and biological activities of the essential oil and anatomical markers of *Lavandula dentata* L. Cultivated In Brazil. Braz Arch Biol Technol. 2018; 61.
7. Budel JM, Wang M, Raman V, Zhao J, Khan SI, Rehman JU, et al. Essential oils of five *Baccharis* species: investigations on the chemical composition and biological activities. Molecules. 2018 Oct; 23(10)2620.
8. Machado CD, Raman V, Rehman JU, Maia BHLNS, Meneghetti EK, Almeida VP, et al. *Schinus molle*: anatomy of leaves and stems, chemical composition and insecticidal activities of volatile oil against bed bug (*Cimex lectularius*). Rev Bras Farmacogn. 2019 Jan-Feb; 29(1): 1–10.
9. Laphitz RML, Semple JC. A Multivariate morphometric analysis of the *Solidago chilensis* Group in South America and related taxa in North America (Asteraceae, Astereae). Ann Mo Bot Gard. 2015 Aug; 100(4): 423–41.
10. Berlyn GP, Miksche JP. Botanical microtechnique and cytochemistry. Iowa: The Iowa State University Press; 1976.
11. Johansen DA. Plant microtechnique. New York: McGraw Hill Book; 1940. 523 p.
12. O'Brien TP, Feder N, McCully ME. Polychromatic staining of plant cell walls by Toluidine Blue O. Protoplasma. 1964 Jun; 59: 368–73.
13. Fuchs CH. Fuchsin staining with NaOH clearing for lignified elements of whole plants or plant organs. Stain Technol. 1963; 38(3): 141–4.
14. Budel JM, Duarte MR, Farago PV, Takeda IJM. Caracteres anatômicos de folha e caule de *Calea uniflora* Less., Asteraceae. Rev Bras Farmacogn. 2006 Mar; 16(1).
15. Budel JM, Duarte MR, Kosciuv I, Morais TB, Ferrari LP. Contribuição ao estudo farmacognóstico de *Mikania laevigata* Sch. Bip. ex Baker (guaco), visando o controle de qualidade da matéria-prima. Rev Bras Farmacogn. 2009 Jun; 19(2b): 545–52.
16. Budel JM, Duarte MR, Farago PV, Franco CRC, Santos VLP, Oliveira AMA. Comparative morpho-anatomical study of *Baccharis curitybensis* Heering ex Malme and *Baccharis spicata* (Lam.) Baill. Lat Am J Pharm. 2011 Nov; 30(8): 1560-6.

17. Budel JM, Duarte MR, Santos, CAM. Caracteres morfo-anatômicos de *Baccharis gaudichaudiana* DC., Asteraceae. Acta Farm Bonaer. 2003; 22(4): 313-20.
18. Souza DMFD, Sá RD, Araújo EL, Randau KP. Anatomical, phytochemical and histochemical study of *Solidago chilensis* Meyen. An Acad Bras Cienc. 2018 Aug; 90(2 suppl 1): 2107–20.
19. Berhin A, Nawrath C, Hachez C. Subtle interplay between trichome development and cuticle formation in plants. New Phytol. 2022 Mar; 233(5): 2036-46.
20. Raeski AP, Heiden G, Novatski A, Raman V, Khan IA, Manfron J. Calcium oxalate crystal macropattern and its usefulness in the taxonomy of *Baccharis* (Asteraceae). Microsc Res Techniq. 2023 Jul; 86(7): 862-81.
21. Meric C, Dane F. Calcium oxalate crystals in floral organs of *Helianthus annuus* L. and *H. tuberosus* L. (Asteraceae). Acta Biol Szeged. 2004 Jan; 48(1): 19–23.
22. Heinrich G, Pfeifhofer HW, Stabentheiner E, Sawidis T. Glandular hairs of *Sigesbeckia jorullensis* Kunth (Asteraceae): morphology, histochemistry and composition of essential oil. Ann Bot. 2002 Apr; 89(4): 459-69.
23. Almeida VP, Hirt AA, Raeski PA, Mika BE, Justus B, Santos VLP, et al. Comparative morphoanatomical analysis of *Mikania* species. Rev Bras Farmacogn. 2017 (Jan-Fev); 27(1): 9-19.
24. Freire SE, Urtubey E, Giuliano DA. Epidermal characters of *Baccharis* (Asteraceae) species used in traditional medicine. Caldasia. 2007 Jun; 29(1): 23–38.
25. Budel JM, Duarte MR. Estudo farmacobotânico de partes vegetativas aéreas de *Baccharis anomala* DC., Asteraceae. Rev Bras Farmacogn. 2008 Dez; 18: 761-68.