



Pharmacognosy

Determination of the metabolic profile of *Solidago canadensis* using UFLC-PDA-ESI-TOF

Andressa Maia Kelly^{1,2}, Temistocles Barroso de Oliveira¹ & Simone Sacramento Valverde^{1,3}

Abstract

Species in the *Solidago* genus are used worldwide due to the therapeutic activities of their flavonoids and terpenoids. Its anti-inflammatory, antirheumatic, analgesic, diuretic, aquaretic, wound-healing properties as well as its ability to relieve burn and insect bites allow use in a variety of situations. This study was carried out to determine the metabolic profile of *Solidago canadensis* inflorescences (SCAI) to identify the species commercialized in Rio de Janeiro, RJ, Brazil, and evaluate the medicinal potential compared to *S. chilensis* and SCAI, which is used in North America and Europe. The UFLC-PDA-ESI-TOF revealed the metabolic profile of SCAI, finding the pseudo-molecular ions and characteristic fragments, mainly flavonols (10) such as quercetin, kaempferol, isorhamnetin, and its glycosides, as well as chlorogenic acid (CA). Eleven substances were identified, confirming the compatibility of the metabolic profile presented in varieties *canadensis* and *scabra* of European *S. canadensis*. The both contain the same flavonoid aglycones, and their glycosides are important chemical and biological markers for extracts and products based on species of the genus *Solidago*. UFLC-PDA-ESI-TOF characterized the extracts, which can help with quality control of raw plant materials and commercialized phytotherapeutics as well as for development of new products and phytomedicines.

Key words: flavonoids, metabolic profile, quality control, *Solidago canadensis*, UFLC-PDA-ESI-TOF.

Resumo

Espécies de *Solidago* são utilizadas mundialmente pelas atividades terapêuticas dos seus flavonoides e terpenoides, ações anti-inflamatória, antirreumática, analgésica, diurética, aquarética, cicatrizante de feridas e queimaduras e em picadas de insetos, que permitem sua utilização em substituição uma à outra. Para determinação do perfil metabólico (PM) de inflorescências de *Solidago canadensis* (SCAI) comercializada no Rio de Janeiro, RJ e avaliação do seu potencial medicinal frente à *S. chilensis* e à SCAI utilizada na América do Norte e Europa, a UFLC-PDA-ESI-TOF é de grande relevância, resolução e rapidez e revelou, através dos íons pseudo-moleculares e fragmentos característicos, o PM de SCAI, formado principalmente flavonóis: quercetina, kaempferol, isoramnetina e seus glicosídeos, além do ácido clorogênico (AC), aos quais são atribuídas importantes atividades biológicas de *S. chilensis*. Onze substâncias, dentre elas 10 flavonóis e o AC, confirmaram a compatibilidade do PM apresentado com as variedades *canadensis* e *scabra* da *S. canadensis* europeia, que apresenta as mesmas agliconas e glicosídeos, marcadores químicos e biológicos para extratos e produtos à base de espécies do gênero *Solidago*. UFLC-PDA-ESI-TOF permite a caracterização de extratos e contribui para o controle da qualidade de matérias-primas vegetais e fitoterápicos comercializados e para o desenvolvimento de novos produtos e fitomedicamentos.

Palavras-chave: flavonoides, perfil metabólico, controle de qualidade, *Solidago canadensis*, UFLC-PDA-ESI-TOF.

¹ FIOCRUZ, Pharmaceutical Technology Institute, R. Sizenando Nabuco 100, 21041-250, Rio de Janeiro, RJ, Brazil.

² ORCID: <<https://orcid.org/0000-0002-4006-811X>>

³ Author for correspondence: simone.valverde@fiocruz.br

Introduction

The ethnopharmacological use of species of the *Solidago* genus has long been used throughout the world and is related to the therapeutic activities of its flavonoids and terpenoids. Some qualitative and quantitative differences exist in anti-inflammatory, bacteriostatic, and aquaretic properties of different goldenrod species (Foster & Tyler 1999; Avila & Fetrow 2000; Robbers & Tyler 2000).

Solidago species are widely used in Europe to treat urinary tract inflammations, preventing formation or facilitating elimination of kidney stones (Robbers & Tyler 2000; Apáti *et al.* 2002; Gastaldi *et al.* 2018). This species naturally occurs throughout the world: *Solidago canadensis* in North America; *Solidago virgaurea* in Europe; *Solidago gigantea* in Asia; and *Solidago chilensis* in South America (Robbers & Tyler 2000; Sutovská *et al.* 2013). These species are present in pharmacopoeias, scientific literature, and pharmaceuticals. *Solidago* is indicated as an anti-inflammatory, antirheumatic, analgesic, diuretic, and aquaretic agent, as well as for healing wounds, burns, and insect bites. The species are often used interchangeably (Robbers & Tyler 2000; Apáti *et al.* 2002; Apáti 2004; Sutovská *et al.* 2013; Oliveira *et al.* 2017; Gastaldi *et al.* 2018; Valverde *et al.* 2012).

In Brazil, *S. chilensis* is the official species of the first edition of FB (Brasil 1926) and on the list of interesting species of the Brazilian public health system (Brasil 2009) with the previous synonymy: *S. microglossa*. It is also on the therapeutic herbal list of many Brazilian cities, such as RJ and SP (SMSDC 2010), while *S. canadensis* is commonly used for ornamental purposes.

To analyze complex plant extracts and fractions, characterizing their contents without isolation of the substances, the metabolomic approach has been used with different chromatographic and spectroscopic techniques.

This work was conducted to determine the metabolic profile of *S. canadensis* inflorescences (SCAI) compared to the officinal Brazilian species *S. chilensis* (SCMI) in order to evaluate their medicinal potential compared to the *S. canadensis* used in North America and Europe and *S. chilensis* used in Brazil.

Material and Methods

Solvents and chemicals

Solidagenone, quercetin, and quercitrin previously isolated from *S. chilensis* inflorescences

and identified through NMR, IR, and MS spectra (Valverde *et al.* 2009; Oliveira & Valverde 2017), and five flavonoids and chlorogenic acid used to investigate the SCAI content and to determine its metabolic profile were the pure standard (Sigma-Merck™). All the solvents used were AR (analytical research grade) or spectral grade according with the analysis.

Plant material and extraction

Solidago canadensis inflorescences were purchased in June 2018 at CADEG (Rio de Janeiro Municipal Market) with the popular description: Tango and the scientific description presumed by the seller as *Solidago canadensis*. The inflorescences (102 g) were dried and pulverized in a knife mill and extracted according with our previous work, with ether: ethanol (1:1), by dynamic maceration, filtered and concentrated to dryness under reduced pressure at 40 °C furnishing 4.1 g of the inflorescences extract (yield 4.2%) (Valverde *et al.* 2009).

Metabolic profile analysis

The TLC analysis was performed using 30 µL aliquots of the raw extract and 10 µL of the pure standard which were eluted with BAW (n-butanol:acetic acid:water - upper phase) (4:1:5) for phenolics and flavonoids and with hexane: ethyl acetate (8:2) for other phytochemicals. The TLCs were physically developed under UV lamp and with reagent solutions, such as NP-PEG for phenolics and flavonoids, to characterize their different phytochemicals using sulfuric anisaldehyde (Wagner & Bladt 1996). TLC plates precoated with silica gel F254 (Merck™) were used.

The UFLC-PDA-ESI-TOF analysis was carried out on a KINETEX™ (100×3×2.6 µm) at 30 °C with a solution of TFA (trifluoroacetic acid) (A) and acetonitrile (B) as mobile phase (flow 0.8 mL/min), in a Shimadzu Nexera 30AD equipped with a quaternary pump, detector with photodiode arrangement and automatic sampler. MS was performed using a Bruker Compact Mass Spectrometer (Bruker™) spectrometer with electrospray ionization interface (ESI) and quadrupole ion trap analyzer to identify directly and simultaneously the presence of flavonoids in the samples, extract, and pure substances. The ESI-MS spectrum was acquired in the negative mode for the sample and reference compounds (Sigma™). Parameters: gas temperature 220 °C,

capillary voltage at 4.5 kV, gas mist (N_2), flow 10 L/min, and pressure at 5.0 Bar. MS was obtained in the full-scan mode in the 50–800 m/z range.

Results and Discussion

The analysis of SCAI extract compounds by UFLC-PDA-ESI-TOF (Fig. 1) revealed its metabolic profile through the pseudo-molecular ions $[M-H]^-$, $[M+Cl]^-$, $[Agly-H-CO_2-CO]^-$, $[Agly-H-CO_2-CO]^-$, $[M+2Cl]^-$, $[M-Rha]^-$, $[M-Gly]^-$, and their characteristic ion fragments.

The structures of these compounds were confirmed by comparing with standard pure compounds such as quercetin, kaempferol, quercitrin, isoquercitrin, and chlorogenic acid, as well as their fragmentation ESI-MS-TOF pattern in experiments and literature data (Saldanha *et al.* 2013; Brito *et al.* 2014; Kim *et al.* 2017; Jang *et al.* 2016).

Only three aglycone, detected by the pseudomolecular anion $[M-H]^-$, quercetin at m/z 301, kaempferol at m/z 285, and isorhamnetin at m/z 315 were identified in the flavonoid content.

All these are flavonols (Tab. 1), and their occurrence was previously reported in the *Solidago* genus (Apáti *et al.* 2002, 2004) and its glycosides, but using HPLC. These flavonoids are responsible to the important biological activities of *S. chilensis*.

The characteristic loss of the sugars in the compounds confirmed the presence of glucose (-162), rhamnose (-146), and rutinose (-308). The identification of all MS detected and identified compounds present in SCAI extract are presented in Figure 2 and Table 1 and explained below.

Identification of compounds

Major compounds detected through the UFLC analysis of the SCAI extract are in Table 1. Peak 1 was identified as chlorogenic acid, λ_{max} 203, 284, and 327 nm, with pseudomolecular anion $[M-H]^-$ at m/z 353 and MS^2 fragment at m/z 161, corresponding to the $[M-192]^-$ loss of quinic acid. Peak 4 was identified as quercetin-3-O- β -D-rutinoside (rutin), with pseudomolecular anion at m/z 609, λ_{max} 255, 353 nm, and MS^2 fragment at m/z 447, corresponding to the $[M-162]^-$ (loss of the glycoside) Gly portion. Peak 5 represented quercetin-3-O- β -D-glycoside (isoquercitrin) $[M-H]^-$ at m/z 463, λ_{max} 207, 309, 256, 266, 352 nm, and MS^2 at m/z 292 corresponding to $[M-171]^-$, a B_2^+ fragment, due to a RDA (Retro Diels-Alder

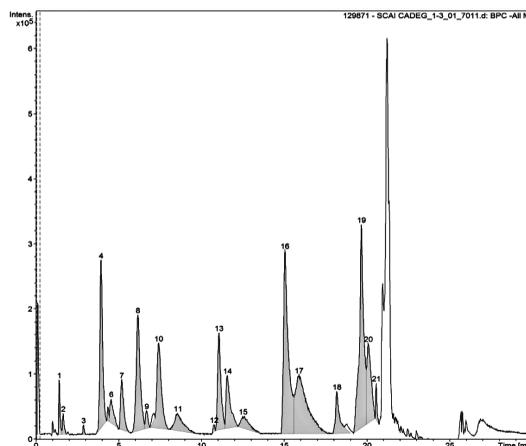


Figure 1 – All MS data, UFLC chromatogram (in negative mode) of phenolic compounds of extract of *S. canadensis* inflorescences.

reaction and MS^3 at m/z 231, corresponding to $[Agly-H-CO_2-CO]^-$, (Agly = aglycone), quercetin (m/z 301). Peak 8 showed a pseudomolecular ion at m/z 593, an adduct $[Agly + 2Cl]^-$ ion at m/z 353 and MS^2 ions at m/z 515: $[M-78]^-$. Peak 10 with a pseudomolecular anion a 505 was identified as quercetin-3-O-6''-acetyl glycoside (MS^2 ions at m/z 587: $[M+81]^-$; 301: $[M-204]^-$, and 431: $[M-74]^-$, respectively). Peak 11, with a pseudomolecular anion at m/z 623 and typical fragments MS^2 at m/z 505: $[M-120]^-$; 431, 283, corresponded to $[M-rut-OCH_3]^-$ and 240. Peak 13 was identified as kaempferol-3-6''-acetyl- β -glycoside with pseudomolecular ions at m/z 489 and MS^2 ions at m/z 571 and 255. Peak 14 indicated a pseudomolecular ion at m/z 519 and an aglycon ion (quercetin) at m/z 301 (MS^2 : $[M-rha-acetyl]^-$), which were identified as quercitrin (quercetin-3-O- β -D-rhamnoside). Peak 16 presented only the pseudomolecular ion $[M-H]^-$ at m/z 285 identified as isorhamnetin with $[M-H]^-$ at m/z 317 and MS^2 ion at m/z 285, corresponding to $[M-CH_3]^-$ (Jang *et al.* 2016; Marczak *et al.* 2016; Pinheiro & Justino 2012; Saldanha *et al.* 2013; March *et al.* 2006).

Conclusions

The UFLC-PDA-ESI-TOF allowed the detection and identification of 13 phenolic compounds in the SCAI extract. Altogether, 11 flavonols were found including: six quercetin derivatives (peaks 4, 5, 6, 9, 10, and 14), three kaempferol derivatives (peaks 8, 13, and 16),

Table 1 – Peak assignments, UV, and MS data (in negative mode) of phenolic compounds extracted from *Solidago canadensis* inflorescences obtained through UFLC-PDA-ESI-TOF.

Peak n°	RT (min)	m/z Max	[M-H] ⁻	UV L _{max} (nm)	Characteristic ion fragments (m/z)	Identification
1	1.4	263.11				NI
2	1.6	353.09	353.09	203, 284, 327	263.11, 161.06	Chlorogenic acid
3	2.9	445.21	-	-	-	NI
4	3.9	609.15	609.15	255, 353	447.10	Rutin (quercetin-3-O-β-D-rutinoside)
5	4.3	463.09	463.09	207, 309, 256, 266, 352	231.97, 292.99	Isoquercitrin (quercetin-3-O-β-D-glucoside)
6	45	609.15	609.15	255, 353	447.10	* Rutin Isomer
7	5.2	515.12	515.12	203, 284, 327	353.09	Chlorogenic acid glucoside
8	6.2	593.16	593.16	207, 326, 238, 469	675.16, 515.12, 353.09, 231.97	Nicotiflorin (kaempferol-3-O-α-L-rutinoside)
9	6.7	447.09	447.09	207, 309, 256, 266, 352	516.13, 353.09, 243.97	*Quercitrin Isomer
10	7.4	505.10	505.10	207, 309, 256, 266, 352	587.10, 431.10	Quercetin-3-O-6''-acetyl glucoside
11	8.6	505.10	623.17	254, 297, 353	505.10, 431.10, 283.27, 240.97	Narcissin (isorhamnetin-3-O-α-L-rhamnopyranosyl-(1→6)-O-β-D-glucopyranoside)
12	10.8	239.16	505.10	-	239.16, 321.17	NI
13	11.1	489.11	489.11	265, 347	571.11, 255.23	kaempferol-3-O-acetyl-β-glucoside
14	11.6	301.04	519.12	207, 255, 350, 469, 672	301.04	Quercitrin (quercetina-3-O-β-D-rhamnside)
15	12.5	301.04	519.12	-	301.04	NI
16	15.0	285.04	285.04	265, 347	-	kaempferol
17	15.9	285.04	315.05	253, 344	285.04	Isorhamnetin
18	18.2	329.23	-	-	-	NI
19	19.7	785.36	-	-	392.17	NI
20	20.1	785.36	-	-	392.17	NI
21	20.6	299.02	-	-	785.36, 601.07, 299.02	NI

* = Not Identified

and two isorhamnetin derivatives (peaks 11 and 17) (Tab. 1), as well as chlorogenic acid and its derivative (peaks 2 and 7).

The results confirmed, with reference substances, fragmentation pattern, UV-vis max, and literature data, that the metabolic profile obtained was similar to the *canadensis* and *scabra* varieties of *S. canadensis* found in Europe, which presents the same flavonoid aglycones and large amounts of their glycosides as described by Apáti (2004).

The UFLC-PDA-ESI-TOF fingerprinting is presently used for rapid quality control purposes to evaluate commercially available medicinal plants widely used by the population, to simultaneously determine their metabolic markers and predict the biological plant performance, their identity, and the confirmation of the metabolic pattern of this plant obtained in a popular market with literature data described for *S. canadensis*.

This work is basis for the quantification of the identified flavonoids, to determine the

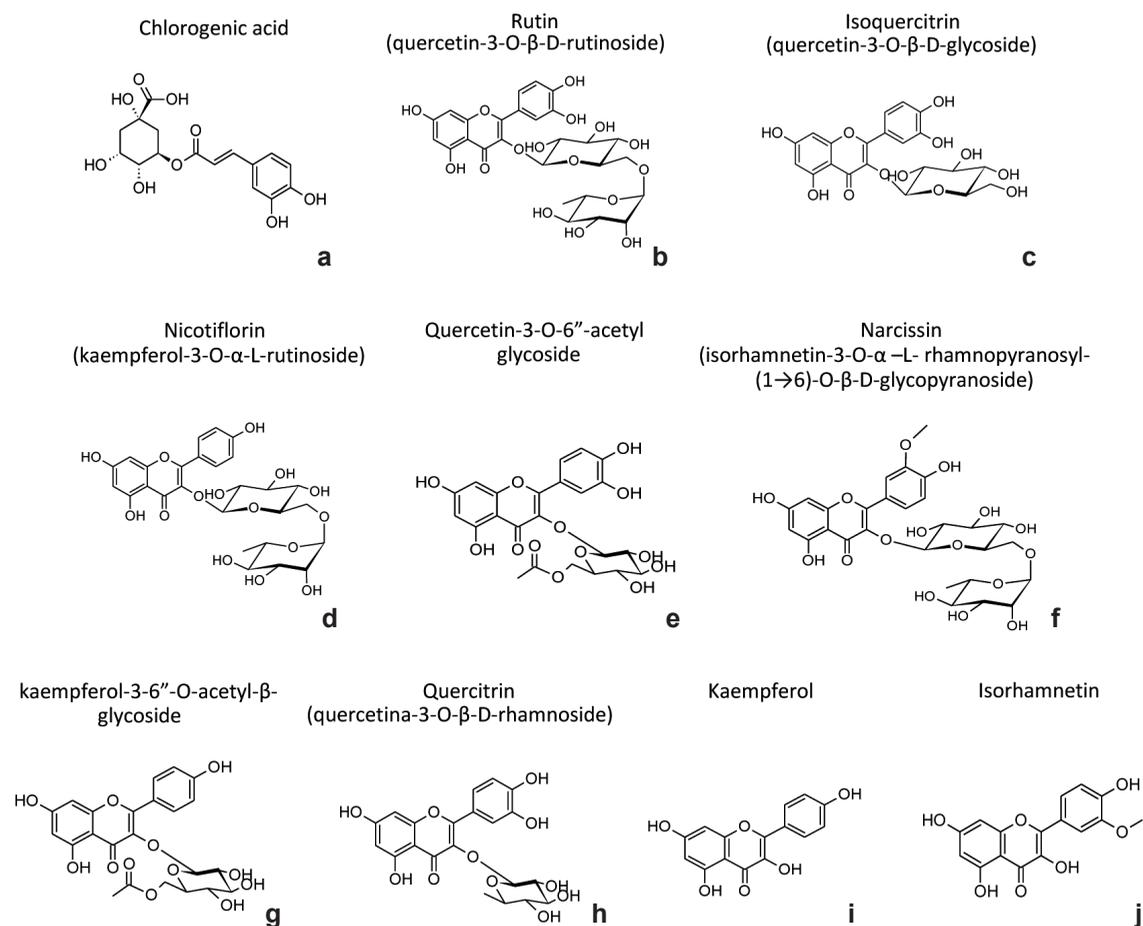


Figure 2 – a-j. Structure of compounds identified in *Solidago canadensis* inflorescences.

biological marker, to the standardization of the marketed SCAI plant extract, and also, to further development of an herbal medicine from SCAI.

Acknowledgements

The authors are grateful to CNPq (National Council for Scientific and Technological Development) for financial support - PROEP and scholarship to AMK.

References

Andersen M & Markham KR (2006) Flavonoids: chemistry, biochemistry and applications. CRC Press Taylor & Francis Group, Boca Raton. 1212p.
 Apáti P, Szentmihályi K, Balázs A, Baumann D, Hamburger M, Kriston TSZ, Szoke E & Kery A (2002) HPLC analysis of the flavonoids in pharmaceutical preparations from Canadian goldenrod (*Solidago canadensis*). Chromatographia Supp. Vol. 56. Springer, Basel. Pp. S65-S68.

Apáti P, Houghton PJ & Kéry A (2004) HPLC investigation of antioxidant components in *Solidago herba*. Acta Pharmaceutica Hungarica 74: 223-31.
 Beck JJ, Smith L & Baig N (2014) An overview of plant volatile metabolomics, sample treatment and reporting considerations with emphasis on mechanical damage and biological control of weeds. Phytochemical Analysis 25: 331-341.
 Brasil - ANVISA (1926) Farmacopeia brasileira. Cia. Editora Nacional, São Paulo. 1149p.
 Brasil - Ministério da Saúde (2009) Relação de plantas de interesse ao SUS. Available at <http://bvsms.saude.gov.br/bvs/sus/pdf/marco/ms_relacao_plantas_medicinais_sus_0603.pdf>. Access on 03 April 2019.
 Brito A, Ramirez JE, Areche C, Sepúlveda B & Simirgiotis MJ (2014) HPLC-UV-MS Profiles of phenolic compounds and antioxidant activity of fruits from three citrus species consumed in Northern Chile. Molecules 19: 17400-17421.
 Eugster PJ, Guillaume D, Rudaz S, Veuthey JL, Carrupt PA & Wolfender JL (2011) Ultra high pressure liquid

- chromatography for crude plant extract profiling. *Journal of Association of Official Analytical Chemists International* 94: 51-70.
- Gastaldi B, Catalán CAN, Silva-Sofrás FM & González SB (2018) *Solidago chilensis* Meyen (Asteraceae), a medicinal plant from South America. A comprehensive review: ethnomedicinal uses, phytochemistry and bioactivity. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* 17: 17-29.
- Jang GH, Kim HW, Lee KM, Jeong YS, Bak AR, Lee DJ & Kim JB (2016) Characterization and quantification of flavonoid glycosides in the *Prunus* genus by UPLC-DAD-QTOF/MS. *Saudi Journal of Biological Sciences* 25: 1622-1631.
- Kim N, Shin YJ, Park S, Yoo G, Kim Y, Yoo HH & Kim SH (2017) Simultaneous determination of six compounds in *Hedera helix* L. Using UPLC-ESI-MS/MS. *Chromatographia* 80: 1025-1033.
- Knox JH (1977) Practical aspects of LC theory. *Journal of Chromatographic Science* 15: 352-364.
- Robbers JE & Tyler E (2000) Tyler's herbs of choice: the therapeutic use of phytomedicinals. The Haworth Press, New York. 312p.
- March RE, Lewars EG, Stadey CJ, Miao X-S, Zhao X & Metcalfe CD (2006) A comparison of flavonoid glycosides by electrospray tandem mass spectrometry. *International Journal of Mass Spectrometry* 248: 61-85.
- Marczak L, Znajdek-Awiżen P & Bylka W (2016) The use of mass spectrometric techniques to differentiate isobaric and isomeric flavonoid conjugates from *Axyris amaranthoides*. *Molecules* 21: 1229.
- Oliveira TB & Valverde SS (2017) Quercetin and quercetin derivatives flavonoids extraction and isolation from *Solidago chilensis* Meyen inflorescences. Vol. 29. Infarma, Brasília. Pp. S577-S577.
- Oliveira TB, Silva FC, Kelly AM & Valverde SS (2017) *Solidago* medicinals. *Revista Brasileira de Plantas Mediciniais* 19: 168-176.
- Pinheiro PF & Justino JC (2012) Structural analysis of flavonoids and related compounds - a review of spectroscopic applications. *Phytochemicals - a global perspective of their role in nutrition and health*. DOI: 10.5772/29152.
- Saldanha LL, Vilegas W & Dokkedal AL (2013) Characterization of flavonoids and phenolic acids in *Myrcia bela* Cambess. Using FIA-ESI-IT-MSⁿ and HPLC-PAD-ESI-IT-MS combined with NMR. *Molecules* 18: 8402-8416.
- SMSDC - Secretaria Municipal de Saúde e Defesa Civil (2010) Prefeitura da Cidade do Rio de Janeiro, Rio de Janeiro. 15p.
- Sutovská M, Capek P, Kocmálová M, Franová S, Pawlaczyk I & Gancarz R (2013) Characterization and biological activity of *Solidago canadensis* complex. *International Journal of Biological Macromolecules* 52: 192-197.
- Valverde SS, Azevedo SS & Tomassini TCB (2009) Utilização de CLAE, como paradigma na obtenção e controle do diterpeno solidagenona a partir de inflorescências de *Solidago chilensis* Meyen (arnica brasileira). *Revista Brasileira de Farmácia* 90: 196-199.
- Valverde SS, Oliveira TB & Souza SP (2012) *Solidago chilensis* Meyen (Asteraceae). *Revista Fitos* 7: 131-136.
- Wolfender JL, Glauser G, Boccard J & Rudaz S (2009) MS-based plant metabolomic approaches for biomarker discovery. *Nat. Prod. Commun.* 4: 1417-1430.
- Wolfender JL, Marti G, Thomas A & Bertrand S (2015) Current approaches and challenges for the metabolite profiling of complex natural extracts. *Journal of Chromatography A* 1382: 136-164.