Mapping Cutaneous Tissue Distribution of Sesquiterpene Lactone Goyazensolide Using MALDI Imaging

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Short Report

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Abstract

Species of *Lychnophora*, popularly known in Brazil as "Arnica-da-serra", are widely used in topical preparations as analgesics and anti-inflammatories. The most commonly used species is *Lychnophora ericoides*, and studies of seasonal and circadian rhythms show greater stability for phenolic constituents than for sesquiterpene lactones (an opposite behavior was observed). These lactones are considered defensive substances; thus, they were found in significant quantities in border regions between two biomes, at least for *L. ericoides*. Herbalists often report that seasonality influences on the increase of lactone levels in leaves and may have increase the allergenic potential. In the present study, we sought to develop a methodology for imaging tissues treated with solutions of the sesquiterpene lactone goyazensolide to observe skin retention using the Franz cell model. To this end, MALDI-MS parameters were optimized for imaging generation, and an LC-MS/MS protocol was used to confirm goyazensolide skin retention. The obtained data revealed that sesquiterpene lactones strongly bind to the skin structure.

Introduction

Species of the genus *Lychnophora* are popularly known in Brazil as “Arnicas-da-serra” or false arnica and are widely collected by herbalists due to their medicinal properties (Cerqueira et al. 1987). *Lychnophora* ericoides is the most used species as an anti-inflammatory and analgesic treatment, which has resulted in a decline in its population (Keles et al. 2010). The main characteristic of these species is its bushy habit that resembles a chandelier, with short, thin leaves and small, superficial roots (Collevatti, Rabelo, and Vieira 2009). Scientifically, the analgesic and anti-inflammatory effects of *L. ericoides* are mainly related to the occurrence of phenolic compounds such as chlorogenic acid (and its derivatives) (Santos et al. 2004; Santos et al. 2005; Moreira et al. 2018), the *C*-flavonoid vicenin (Gobbo-Neto et al. 2005; Silva et al. 2014), lignans (Borsato et al. 2000) and monoterpenes (Pavarini et al. 2013). All activities were demonstrated *in vivo* and with well-defined mechanisms of action, but for other *Lychnophora* species sesquiterpene lactones are also related to the anti-inflammatory activity (Santos et al. 2010; Marinho et al. 2023). Recently, Marinho and co-workers (2023) showed that topical treatment with ethanolic extracts of *L. ericoideis* leaves (with phenolic compounds as the major compounds) increases important factors for wound healing such as collagen formation and the expression of the proliferative axis of the renin-angiotensin system. Generally, the *in vivo* anti-inflammatory action is attributed to the sesquiterpene lactones (Ugoline et al. 2017). Sesquiterpene lactones isolated from other *Lychnophora* species exhibit an anti-inflammatory effect by neutrophil migration inhibition and TNF-α production in addition to antinociceptive activity (Bernardes et al. 2021). However, in cellular models, the binding of goyazensolide derivatives with the cysteine residue of glutathione was observed through a reaction similar to Michael's addition. This depletion of glutathione in the inflammatory process can lead to greater oxidative stress and thus present an effect that is antagonistic to what was expected.

A recent review discusses that sesquiterpene lactones may be considered the main allergens of plants in the *Asteraceae* family (Paulsen 2023). Currently, the average prevalence of allergy reactions to these

compounds in Europe is around 1%, and in countries outside Europe and North America, it can reach close to 11%. This information suggests the need for ongoing studies on potential adverse reactions of this class of secondary metabolites (Paulsen 2023). In Brazil, herbalists and collectors report that at a specific period of the year, the leaves of *L. ericoides* show better biological activity than in other months and the less intensive activity shows an increase in the allergenic potential in the skin (Semir et al. 2011), but, until now there are no data about the skin absorption of sesquiterpene lactones.

In the skin, three distinct routes to cross the stratum corneum are intercellular, transcellular, and through cutaneous appendages (Gratieri, Gelfuso, and Lopez 2008). Different topical application tests are utilized to investigate the penetration profile of natural products into the skin, as exemplified by the use of *L. ericoides* hydrolate (Gratieri, Gelfuso, and Lopez 2008; Semir et al. 2011). The application of these models yields results on the penetration profile, the permeation route, the mechanism of action of carrier systems, and the determination of the substance in the different layers of the skin (Depieri et al. 2015). The most commonly used model is the Franz cell. In this model, the substance is initially applied to a donor compartment in contact with a receptor compartment where the diffusion process occurs. The subsequent compartment is referred to as the receptor and donor, housing the membrane that acts as a barrier to the penetration of substances (Franz 1975). Electrospray ionization mass spectrometry (ESI) hyphenated with liquid chromatography (LC) is commonly used for the quantitative analysis of permeation in the carrier liquid (Pafili et al. 2021) however, mass spectrometry imaging allows us to understand more about chemical distribution through image generation (Handler et al. 2020). In this way, these model's applications may be useful to clarify skin absorption of natural products.

It is important to reinforce that sesquiterpene lactones of *L. ericoides* can be not observed in all plants during different seasons. Analysis of the circadian and seasonal rhythm of the secondary metabolites of *L. ericoides* indicated that during the best collection period, there are the lowest concentrations of lactones (Sakamoto et al. 2003). When plants are on the border between two biomes, like Cerrado (Brazilian Savanna) and Atlantic Forest, the production and accumulation of these sesquiterpene lactones is amplified (Gobbo-Neto et al. 2010), which reinforces a strong correlation of defense against possible environmental effects (Ahern and Whitney 2014). As already mentioned for some species of *Lychnophora*, such as *L. passerine* and *L. trichocarpha*, the activity is directly related to the presence of lactones (Bernardes et al. 2021) nevertheless, in *L. salicifolia*, popularly known as “Arnicão”, there are no reports of accumulations of lactones, only the same previous discussed phenolic active compounds. Hence, given the comprehensive understanding of the biological activity within the genus, a pivotal inquiry arises: Are the sesquiterpene lactones from L. ericoides capable of being absorbed and uniformly distributed through the skin, or are they entirely absorbed into the bloodstream and subsequently eliminated via renal pathways? To address this query, the present study devised analytical methodologies employing LC-MS/MS and MALDI-imaging analysis of goyazensolide within a Franz cell model.

Material and methods

General experimental procedures

1D NMR experiments (^{1}H, HSQC, and HMBC) were performed in $CDCl_3$ on a Bruker DRX-600 spectrometer (Bruker BioSpin, GmBH) equipped with a Bruker 5-mm TCI CryoProbe. UPLC-MS/MS separation and analysis were performed in ACQUITY TQD UPLC-MS (Waters®) and HRMALDI-MS were acquired in the positive ion mode on an UltrafleXtreme (BrukerDaltonics®) equipped with a MALDI ionization source (smart beam type laser).

Plant material

The leaves of *L. ericoides* were collected in February 2012 in the city of Ibiraci (S 20°20'046', W 047°08'229"; 1090 m altitude, Ibiraci, Minas Gerais State) and identified by Professor João Semir. A voucher specimen has been deposited at the Unicamp Herbarium (NPL284). Leaves of *E. mattogrossensis* were also collected at Ibiraci in March 2012 and identified by Professor João Semir. A voucher specimen has been deposited at the Unicamp Herbarium (NPL-EM).

Extraction and isolation

Air-dried intact leaves of *L. ericoides* (0,1 kg), collected in Ibiraci growing in a visible transitional zone between the typical "Campos Rupestres" and a semi-deciduous forest were rinsed with 0,4 L of CH_2Cl_2 for 10 min at room temperature (27°C). After filtration, the crude extract was dried under reduced pressure at 50°C and analyzed to confirm the presence of goyazensolide. The air-dried leaves (*Eremanthus mattogrossensis* 1 kg) were rinsed with 4L of CH_2Cl_2 for 10 min at room temperature (27°C) to increase the amount of goyazensolide. The CH_2Cl_2 extract was passed through a paper filter and concentrated under reduced pressure at 50°C to give 60g (6%) of the crude extract. An aliquot of this crude extract (20 g) was submitted to classic chromatography of the short column model—SCM on silica gel 60 as previously described (Sousa et al. 2012), which yielded 200 mg of the pure lactone.

UPLC-MS/MS analysis

An ACQUITY TQD UPLC-MS Waters® equipment was used to determine goyazensolide in the receptor medium and skin samples. The chromatographic separation was carried out with a Kinetex 1.7 µm C18 column with dimensions of 2.1 x 50 mm maintained at 40°C. The mobile phase used was water (A) and methanol (B), both with 0.1% formic acid at a flow rate of 0.3 mL.min-1 in a gradient mode as shown in the supplementary material in Table S1. Electrospray source detection was acquired in MRM (multiple reaction monitoring) mode and with positive ionization. The precursor ion fragmentation at *m/z* 361 was monitored using the fragment at *m/z* 275 previously described (Crotti, Lopes, and Lopes 2005). The energy applied to the extractor cone was 25V, and the collision gas energy was 10 eV. Full details of the mass detector parameters are found in the supplementary material Table S2. The skin samples were obtained from sections of the same tissue used for the MALDI-MSI analyses to provide greater confidence in the analyses. Each sample was transferred to a 2 mL Eppendorf tube, and then 500 µL of methanol: water (1:1 *v/v*) solvent mixture was added. The samples were homogenized with the aid of the T10 Ultra Turrax homogenizer (IKA®), and after the end of the process, they were placed in an ultrasound

bath for 10 min and subsequently centrifuged for 10 min at 4°C and 15000 x g. After centrifugation, 250 µL of the supernatant was transferred to smaller volume vials, and 5 µL was injected into the UPLC.

MALDI-imaging and tissue preparations

The goyazensolide compound was solubilized in methanol: water (8:2 *v/v*) and homogenized in a 1:1 ratio with different matrices (2.5 dihydroxybenzoic acid, α-cyano-4-hydroxycinnamic acid, synaptic acid, and caffeic acid) to select the best metrices, and transferred to a metal plate (Bruker®) in different concentrations. Furthermore, the addition of NaCl favors the sodiated ion and TFA for the protonated ion evaluation. The instrumental conditions used to obtain the spectra were 500 laser shots per spectrum, PIE (pulsed ion extraction) of 130 ns, positive mode, and laser frequency of 1000 Hz. The IS1 voltage (ion source 1) was 25.00 kV, and the IS2 (ion source 2) was 22.55 kV. The reflector mode was used with voltage applications at RV1 (reflector) and RV2 of 26.60 kV and 13.35 kV, respectively. The laser intensity was varied to better obtain the $[M + H]^+$ and $[M + Na]^+$ ions of the analytes in each matrix. The mixture of flavonoids (galangin, quercetin, isoquercetin, and rutin) was used for external calibration of the equipment. The images were acquired with a spatial resolution of 50 µm.

For tissue analysis, pig ear skins (commercially available from a small local farmer) were used for the distribution assay (blank, control, 1h, 3h, and 6h). Skins were transversely sectioned with a thickness of 12 µm, using a Leica CM1860 rotary cryostat (Leica®) operating at − 25°C. The sections were placed on conductive slides coated with indium tin oxide (Bruker®) and kept at -60°C until analysis. Slides were also prepared with the aid of double-sided carbon adhesive tape to ensure the fixation of the skins (Goodwin et al. 2012). Serial sections were placed on slides for histology, which were later stained with hematoxylin and eosin for histological analysis of the tissue. The images were obtained using a Leica DM 4000B microscope coupled to a DFC500 scientific digital camera, using the LAS V3.8 program to acquire the images.

To evaluate the influence of the biological matrix on the ionization of substances in the skin, white skin samples were contaminated with substances in concentrations ranging from 0.5 to 2000 $\mu g.mL^{-1}$. The previously selected matrix was applied to these contaminated samples with the help of ImagePrepTM from Bruker®, a device that alternates cycles of matrix application and drying (incubation) with an N_2 atmosphere. The slides were then placed in a slide adapter for MALDI-MSI analysis. Analysis of the data obtained and generation of images were carried out using FlexImaging software (Bruker®).

From the MALDI-MSI analyses carried out with contaminated skin samples, it was possible to make adjustments to both sample preparation and composition and proportion of the extracting solvent, matrix concentration, application cycle, incubation, drying in ImagePrep, and spectrum acquisition. And so, the established procedure was applied to the samples obtained in in vitro distribution assays using Franz cells.

Results and Discussion

Initially, air-dried intact leaves of *L. ericoides*, collected in Ibiraci, which grows in a visible transitional zone between the typical "Campos Rupestres" and a semi-deciduous forest, were subjected to isolated goyazensolide. As previously described, *L. ericoides* is a vulnerable species, and the necessity to obtain it on a larger scale prompted us to conduct fractionation of an alternative species *Eremanthus mattogrossensis*, an arboreal plant that provides greater biomass. Firstly, we determine the goyazensolide chemical structure, which was performed by analysis of the MS fragmentation pattern (Crotti, Lopes, and Lopes 2005) and the NMR data comparison with those reported in the literature (Cunha et al. 1995). Copies of the original spectra are obtainable from the corresponding author. NMR spectra of goyazensolide and MS/MS data are available at Supporting Information.

The subsequent stage involved examining the ionization reactions of goyazensolide in the MALDI-MS system to comprehend the potential equilibrium between charge generation reactions. Among the matrices tested, the combination of HCCA + DHB in a 1:1 ratio in methanol: water (8:2, *v/v*) at a concentration of 10 mg/mL containing 0.25 mg/mL NaCl exhibited the most efficient charge transfer system. Under these conditions, the unexpected $[M + Na]^+$ ion (*m/z* 383) appeared more prominently (Fig. 2A), even in the absence of added sodium. Furthermore, the addition of diluted acid failed to reverse this process. Two phenomena could contribute to this effect: the availability of sodium ions in the physiological environment and the analyte's chemical structure, which exhibits significant coordination ability, as commonly observed in secondary metabolites (Silva, Lopes, and Silva 2016; Lopes et al. 2001). Another less-discussed aspect is how the addition of a phase modifier or increased energy can generate reaction artifacts at the source, resulting in expected *m/z* values and potentially leading to erroneous analyses, as observed with natural ionophores (Lopes et al. 2002). In a recent literature review, Silva and collaborators (2016) present that several natural products can generate, in the MALDI source, ions that do not occur in ESI, corroborating our proposal to develop the methodology monitoring the $[M + Na]^+$ ion.

Opting to monitor the sodiated molecule, we conducted laser scanning analysis of the histological sections from the Franz cell tract. Across all repetitions, the images consistently depicted an accumulation of sesquiterpene lactone, primarily concentrated in the region of the stratum corneum (see Fig. 2B). It's worth emphasizing that the stratum corneum serves as a natural skin barrier, presenting one of the principal challenges in studies concerning skin distribution (Gratieri, Gelfuso, and Lopez 2008). Many substances have difficulties in overcoming this barrier. The main component of this layer is keratin, which has cysteine, among several amino acids in its composition. The sesquiterpene lactones have an α-β-unsaturated carbonyl group (present in the lactone ring) reacts with nucleophilic groups, mainly cysteine sulfhydryl residues, through Michael addition (Rüngeler et al. 1999). The combined analysis of this data suggests that the predominant accumulation, through interactions with skin structures, may contribute to undesired effects. Additional analyses using UPLC-MS/MS confirm the existence of covalent bonds, providing insights into the levels of goyazensolide in both tissues and receptor media.

Conclusions

The seasonal variations described for the sesquiterpene lactones of *L. ericoides* and the evidence presented follow the popular description confirming that the topical uses of ethanol/water solutions may present - in some months - a topical allergenic reaction at the point of application.

In conclusion, the UPLC-MS/MS analyses provided valuable insights into the presence of goyazensolide in pig ear skin and the Franz cell receptor medium. These findings corroborated the data obtained from MALDI-MSI, underscoring the significance of our results. Moreover, the observed seasonal variations in sesquiterpene lactones of *L. ericoides* align with the popular application, confirming that ethanol/water solutions used topically may elicit allergenic reactions at the application site during certain months. This highlights the importance of considering seasonal factors in the development and use of topical formulations containing sesquiterpene lactones. Further studies are warranted to elucidate the underlying mechanisms and optimize the safety and efficacy of such formulations.

Declarations

Supporting Information

NMR, MS and MS/MS spectra of goyazensolide in addition to LC-MS/MS analysis are present as Supporting Information.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

NPL was responsible to conceptualization, experimental design, data analysis, writing, reviewing and editing the manuscript, and funding research. GAB was responsible for the conceptualization, experimental design, performing experiments and data analysis, reviewing, and editing the manuscript; NNK contributed to data analysis, writing, reviewing, and editing the manuscript; JNM conducted the experiments, obtained the MALDI data, and data analysis; JLCL conducted the data analysis, writing and reviewing; RFVL performed the skin experiments and data analysis.

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References

1. Ahern JR, and Whitney, KD (2014) Sesquiterpene lactone stereochemistry influences herbivore resistance and plant fitness in the field. Ann Bot. 113 (4): 731–40. https://doi.org/10.1093/aob/mct297.
2. Bernardes ACFPF, Matosinhos RC, Araújo MCPM, Barros CH, Soares RDOA, Costa DC, Sachs D, and Saúde-Guimarães DA (2021) Sesquiterpene lactones from *Lychnophora* species: antinociceptive, anti-inflammatory, and antioxidant pathways to treat acute gout. J Ethnopharmacol. 269: 113738. https://doi.org/10.1016/j.jep.2020.113738.
3. Borsato MLC, Grael CFF, Souza GEP, and Lopes NP (2000) Analgesic activity of the lignans from *Lychnophora ericoides*. Phytochem. 55 (7): 809–13. https://doi.org/10.1016/S0031-9422(00)00388-5.
4. Cerqueira MBS, Souza JT, Amado Júnior R, and Peixoto ABF (1987) Ação analgésica do extrato bruto aquoso liofilizado do caule e folhas da *Lychnophora ericoides* Mart. (Arnica). Ciênc. Cult. (São Paulo) 39 (5/6): 551–53. https://pesquisa.bvsalud.org/portal/resource/pt/lil-43900.
5. Collevatti RG, Rabelo SG, and Vieira RF (2009) Phylogeography and disjunct distribution in *Lychnophora ericoides* (Asteraceae), an endangered cerrado shrub species. Ann of Bot. 104 (4): 655–64. https://doi.org/10.1093/aob/mcp157.
6. Crotti AEM, Lopes JLC, and Lopes NP (2005) Triple quadrupole tandem mass spectrometry of sesquiterpene lactones: a study of goyazensolide and its congeners. J Mass Spectrom. 40 (8): 1030–34. https://doi.org/10.1002/jms.877.
7. Cunha WR, Lopes JLC, Vichnewski W, Díaz JG, and Herz W (1995) Eremantholides and a Guaianolide from *Lychnophora rupestris*. Phytochem. 39 (2): 387–89. https://doi.org/10.1016/0031-9422(94)00945-P.
8. Depieri LV, Praça FSG, Campos PM, and Bentley MVLB (2015) Advances in the bioanalytical study of drug delivery across the skin. Ther Deliv. 6 (5): 571–294. https://doi.org/10.4155/tde.15.20.
9. Franz TJ (1975) Percutaneous absorption. on the relevance of *in vitro* data. J Invest Dermatol. 64 (3): 190–95. https://doi.org/10.1111/1523-1747.ep12533356.
10. Gobbo-Neto L, Guaratini T, Pessoa C, Moraes MO, Costa-Lotufo LV, Vieira RF, Colepicolo P, and Lopes NP. (2010) Differential metabolic and biological profiles of *Lychnophora ericoides* Mart. (Asteraceae) from different localities in the Brazilian 'Campos Rupestres.' J Braz Chem Soc. 21 (4): 750–59. https://doi.org/10.1590/S0103-50532010000400023.
11. Gobbo-Neto L, Santos MD, Kanashiro A, Almeida MC, Lucisano-Valim YM, Lopes JLC, Souza GEP, and Lopes NP (2005) Evaluation of the anti-inflammatory and antioxidant activities of di-*C*-glucosylflavones from *Lychnophora ericoides* (Asteraceae). Planta Med. 71 (1): 3–6. https://doi.org/10.1055/s-2005-837742.
12. Goodwin RJA, Nilsson A, Borg D, Langridge-Smith PRR, Harrison DJ, Mackay CL, Iverson SL, and Andrén PE (2012) Conductive carbon tape used for support and mounting of both whole animal and

fragile heat-treated tissue sections for MALDI MS imaging and quantitation. J Proteom. 75 (16): 4912–20. https://doi.org/10.1016/j.jprot.2012.07.006.

13. Gratieri, T, Gelfuso GM, and Lopez RFV (2008) Princípios básicos e aplicação da iontoforese na penetração cutânea de fármacos. Quim Nova. (31): 1490-1498. https://doi.org/10.1590/S0100-40422008000600040.
14. Handler AM, Fallah M, Pedersen AJ, Pedersen GP, Nielsen KT, and Janfelt C (2020) MALDI mass spectrometry imaging as a complementary analytical method for improved skin distribution analysis of drug molecule and excipients. Int J Pharm. 590: 119949. https://doi.org/10.1016/j.ijpharm.2020.119949.
15. Keles LC, Melo NI, Aguiar GP, Wakabayashi KAL, Carvalho CE, Cunha WR, Crotti AEM, Lopes JLC, and Lopes NP (2010) Lychnophorinae (Asteraceae): A survey of its chemical constituents and biological activities. Quim Nova. 33 (10): 2245–60. https://doi.org/10.1590/S0100-40422010001000038.
16. Lopes NP, Stark CBW, Hong H, Gates PJ, and Staunton J (2001) A study of the effect of pH, solvent system, cone potential and the addition of crown ethers on the formation of the monensin protonated parent ion in electrospray mass spectrometry. Analyst. 126 (10): 1630–32. https://doi.org/10.1039/b106852k.
17. Lopes NP, Stark CBW, Gates PJ, and Staunton J (2002) Fragmentation studies on monensin A by sequential electrospray mass spectrometry. Analyst. 127 (4): 503–6. https://doi.org/10.1039/b110412h.
18. Marinho BM, Guimarães VHD, Moraes DS, Ribeiro GHM, Silva RM, Lopes NP, Guimarães ALS, Paula AMB, and Santos SHS (2023) *Lychnophora ericoides* Mart. (Brazilian Arnica) ethanol extract accelerates the skin wound healing process: evidence for its mechanism of action. Phytomedicine. 119: 155000. https://doi.org/10.1016/j.phymed.2023.155000.
19. Marinho BM, Lescano CH, and Santos SHS (2023) Brazilian Arnicas: bioactive compounds, pharmacological properties, potential use and clinical applications. Phytochem Rev. 0123456789. https://doi.org/10.1007/s11101-023-09884-x.
20. Moreira EA, Pilon AC, Andrade LE, and Lopes NP (2018) New perspectives on chlorogenic acid accumulation in harvested leaf tissue: impact on traditional medicine preparations. ACS Omega. 3 (12): 18380–86. https://doi.org/10.1021/acsomega.8b02409.
21. Pafili A, Meikopoulos T, Kontogiannidou E, Papageorgiou S, Demiri E, Meimari D, Fatouros DG, Gika H, and Theodoridis G (2021) Development and validation of LC-MS/MS method for the determination of UV-filters across human skin *in vitro*. J Chromatogr B Analyt Technol Biomed Life Sci. 1167: 122561. https://doi.org/10.1016/j.jchromb.2021.122561.
22. Paulsen E (2023) The sesquiterpene lactone mix: A review of past, present and future aspects. Contact Dermatitis. 89 (6): 434–41. https://doi.org/10.1111/cod.14419.
23. Pavarini DP, Nogueira EF, Callejon DR, Soares DM, Souza GEP, Cunha FQ, Lopes JLC, and Lopes NP (2013) Novel bisabolane derivative from 'Arnica-Da-Serra' (Vernonieae: Asteraceae) reduces pro-

nociceptive cytokines levels in LPS-stimulated rat macrophages. J Ethnopharmacol. 148 (3): 993–98. https://doi.org/10.1016/j.jep.2013.05.003.

24. Rüngeler P, Castro V, Mora G, Gören N, Vichnewski W, Pahl HL, Merfort I, and Schmidt TJ (1999) Inhibition of transcription factor NF-KB by sesquiterpene lactones: A proposed molecular mechanism of action. Bioorg Med Chem. 7 (11): 2343–52. https://doi.org/10.1016/S0968-0896(99)00195-9.
25. Sakamoto HT, Flausino D, Castellano EE, Stark CBW, Gates PJ, and Lopes NP (2003) Sesquiterpene lactones from *Lychnophora ericoides*. J Nat Prod. 66 (5): 693–95. https://doi.org/10.1021/np020314v.
26. Santos MD, Chen G, Almeida MC, Soares DM, Souza GEP, Lopes NP, and Lantz RC (2010) Effects of caffeoylquinic acid derivatives and *C*-flavonoid from *Lychnophora ericoides* on *in vitro* inflammatory mediator production. Nat Prod Commun. 5 (5): 733–40. https://doi.org/10.1177/1934578x1000500512.
27. Santos MD, Gobbo-Neto L, Albarella L, Souza GEP, and Lopes NP (2005) Analgesic activity of di-caffeoylquinic acids from roots of *Lychnophora ericoides* (Arnica da serra). J Ethnopharmacol. 96 (3): 545–49. https://doi.org/10.1016/j.jep.2004.09.043.
28. Santos PA, Amarante MFC, Pereira AMS, Bertoni B, França SC, Pessoa C, Moraes MO, Costa-Lotufo LV, Pereira MRP, and Lopes NP (2004) Production of an antiproliferative furanoheliangolide by *Lychnophora ericoides* cell culture. Chem Pharm Bull. 52 (12): 1433–35. https://doi.org/10.1248/cpb.52.1433.
29. Semir J, Rezende AR, Monge M, and Lopes NP (2011) As Arnicas endêmicas das serras do Brasil. Ouro Preto: Editora UFOP.
30. Silva DB, Turatti ICC, Gouveia DR, Ernst M, Teixeira SP, and Lopes NP (2014) Mass spectrometry of flavonoid Vicenin-2, based sunlight barriers in *Lychnophora* species. Sci Rep. 4 (1), 4309. https://doi.org/10.1038/srep04309.
31. Silva R, Lopes NP, and Silva DB (2016) Application of MALDI Mass Spectrometry in natural products analysis. Planta Med. 82 (8): 671–89. https://doi.org/10.1055/s-0042-104800.
32. Sousa JPB, Tomaz JC, Silva CCP, Ellena J, Lopes NP, and Lopes JLC (2012) Novel sesquiterpene lactones from *Eremanthus seidelii*. Tetrahedron Lett. 53 (47): 6339–42. https://doi.org/10.1016/j.tetlet.2012.09.007.
33. Ugoline BCA, Souza J, Ferrari FC, Ferraz-Filha ZS, Coelho GB, and Saúde-Guimarães DA (2017) The influence of seasonality on the content of goyazensolide and on anti-inflammatory and anti-hyperuricemic effects of the ethanolic extract of *Lychnophora passerina* (Brazilian Arnica). J Ethnopharmacol. 198: 444–50. https://doi.org/10.1016/j.jep.2017.01.017.

Figures

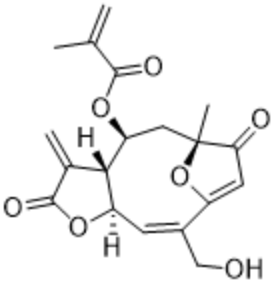

Figure 1

Chemical structure of goyazensolide

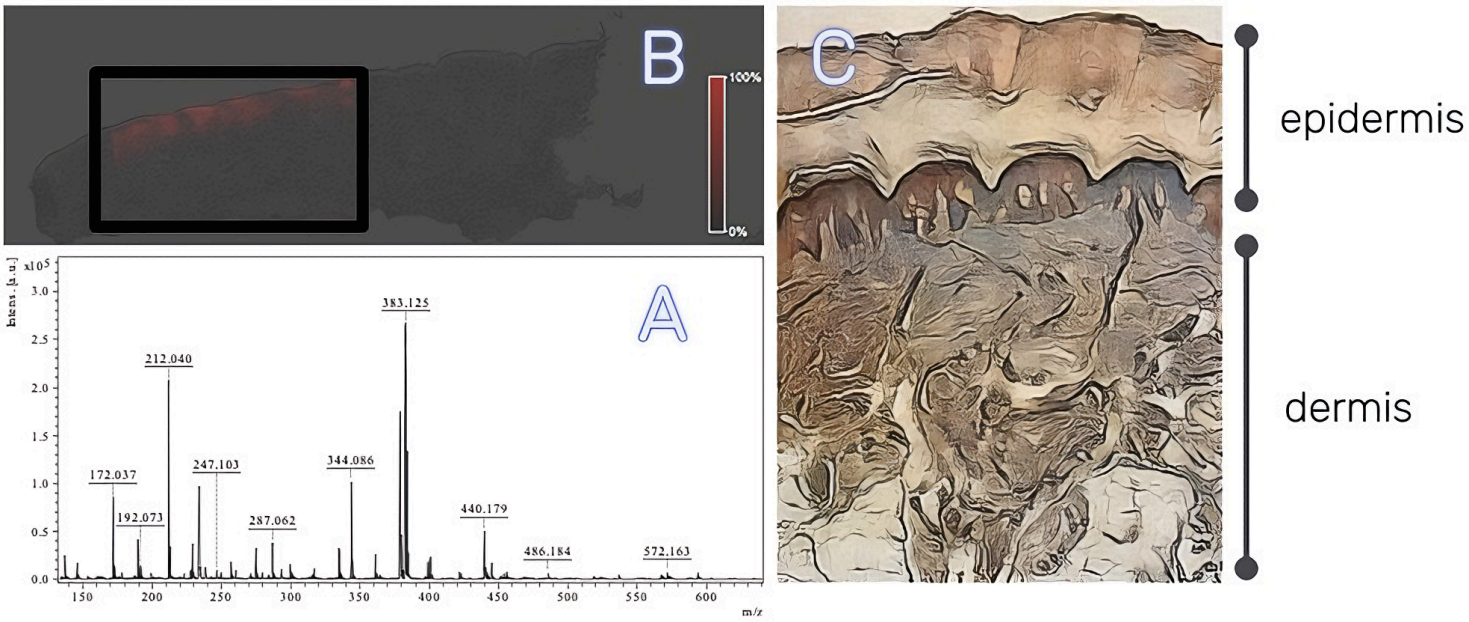

Figure 2

MALDI-imaging analysis. A) MS spectra of the goyazensolide showing the sodium adduct; B) Tissue analysis demonstrating the distribution region of the goyazensolide; C) Skin details for comparison of each single region.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SM.docx
- Slide1.tiff