Phytochemical Profiling of Aloe koenenii leaf epidermis by UHPLC-PDA-MS/MS

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Short Report

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Abstract

Aloe koenenii is an understudied *Aloe* species, and its leaf-epidermis chemical components has not yet been characterized. In this work, we provide the first characterization of the leaf-epidermis metabolite profile of *A. koenenii* and compare it with *Aloe vera* using UPLC–PDA–MS/MS. Chromatographic peaks were evaluated by UV–Vis absorption patterns and MS/MS fragmentation, enabling putative annotation of key constituents. Four compounds were annotated in *A. koenenii*: aloin A, aloin B, aloesin, and 7-hydroxy-8-*O*-methylaloin. All four were also detected in *A. vera*, which additionally showed three further annotated constituents: homonataloside B, 7-hydroxyaloin A or B, and isoaloeresin D or aloeresin D. Thus, the two species share a core set of anthrone- and chromone- related metabolites, while *A. vera* exhibited a more diverse profile under the conditions evaluated. These data support chemotaxonomic affinity within the genus and highlight candidate marker compounds that may assist quality control and authentication of *Aloe* leaf materials, providing a chemical basis for future targeted bioactivity studies.

Introduction

Aloe (Asphodelaceae) is a genus of succulent, perennial, and drought-resistant plants, comprising more than 400 species distributed across Africa, the Arabian Peninsula, India, and other arid regions (Diriba and Deresa, 2022). Their leaves have been used for therapeutical applications (burns, wounds, ulcers, constipation, cough, and diabetes treatment) (Benítez et al. 2015). Several components have been identified in the leaves of *Aloe* species, including anthraquinones, anthrones, chromones, phenyl pyrones, naphthalene derivatives, and mucilages. The main compounds in *Aloe* leaf epidermis are aloin A and B (anthrone glycosides), aloeresin A, aloesin A and B (chromone glycosides) (Wu et al. 2013; Zhong et al. 2015).

Aloe koenenii Lavranos & Kerstin Koch Lodé (Figure **S1**) is found in Jordan and differs from *Aloe vera* (L.) Burm.f. by its red flowers instead of yellow and its inability to produce seeds (Lavranos and Koch, 2006). *A. vera* is the main *Aloe* species and several studies have demonstrated the pharmacological activities of its leaves. The leaf mucilaginous gel has well-established anti-inflammatory and wound-healing properties and is used as a topical agent for the treatment of burns, wounds, and inflammatory processes, while the leaf epidermis has a laxative effect due to the presence of anthraquinones and anthrones (Kumar et al. 2024)

Despite extensive research on *Aloe* species, the chemical composition of *A. koenenii* remains undocumented. Here, we analyze its leaf epidermis by UHPLC-PDA-MS/MS and compare it with *A. vera* leaf epidermis chemical composition.

Materials and Methods

Plant material and extract

A. koenenii leaves were collected at Registro, state of São Paulo, Brazil (24º26’12,261” S, 47º53’34,905” W) in October 2017, while *A. vera* leaves were collected at the Medicinal Botanical Garden of the School of Pharmaceutical Sciences of UNESP, Araraquara, Brazil (21º48’52,9” S, 48º12’06,8” W) in January 2019. The plant material was identified by Dr. Luis V. S. Sacramento (School of Pharmaceutical Sciences, UNESP) and voucher specimens (*A. koenenii* 001 and *A. vera* 002) were deposited at the Herbarium “São José do Rio Preto” of the São Paulo State University - UNESP, Brazil.

The leaf epidermis was separated and dried in an air circulation oven at 40°C for 3 days and subsequently powdered using a knife mill. Dried and powdered epidermis (50.0 g) was first extracted with hexane (250 ml) by maceration at room temperature (4 h) to remove low-polarity compounds (interferents). The phenolic compounds were extracted with ethanol (250 ml) by maceration at room temperature, in the absence of light, under occasional stirring in 3 steps (8, 12, and 24 h). The three extractive solutions were combined, filtered, and the solvent was removed under reduced pressure

using an IKA DEST KV 05S3 rotary evaporator to yield 7.6 and 4.0 g of ethanolic dry extracts of *A. vera* and *A. koenenii*, respectively.

Ultra-High Performance Liquid Chromatography-Photodiode Array Detector-Mass Spectrometry (UHPLC-PDA-MS/MS) analysis

Extracts were prepared at 10.0 mg/ml in methanol:water (98:2, v/v) and subjected to solid-phase extraction using a Strata™ C18 cartridge (1.5 × 1.0 cm, 55 µm; Phenomenex®), preconditioned and eluted with 5.0 ml of methanol:water (98:2, v/v). Chromatographic analyses were performed on an ACQUITY UPLC H-Class system (Waters®) coupled to a photodiode array detector and a Xevo TQ-S triple quadrupole mass spectrometer (Waters® Corporation, Milford, MA) equipped with a Z-spray ion source. Separation was achieved on an Ascentis® Express C18 column (100 mm × 4.6 mm, 2.7 µm; Supelco®). Aliquots of 5.0 µl (100 µg/ml in methanol) were injected. The mobile phase consisted of water containing 0.1% formic acid (solvent A) and methanol containing 0.1% formic acid (solvent B). The gradient program was as follows: 30% B increased to 50% B over 0–10 min; raised to 85% B from 10–15 min; held for 1 min; and subsequently returned to initial conditions (30% B) for 5 min, resulting in a total runtime of 22 min. The flow rate was maintained at 0.40 ml/min. UV–Vis spectra were recorded between 200 and 700 nm. Mass spectrometric detection was conducted under electrospray ionization in both positive and negative modes. Capillary voltages were set at 3.2 kV (positive) and 2.5 kV (negative), with a cone voltage of 40 V. The ion source temperature was 150°C, and desolvation was performed using nitrogen (99.9% purity) at 300°C with a gas flow of 350 l/h. Data were acquired over an m/z range of 100–800. Collision-induced dissociation experiments employed argon (99.9999% purity) as the collision gas. Data were acquired and processed using MassLynx® 4.1 software. PDA chromatograms recorded in full-scan mode (200–700 nm) for *A. vera* and *A. koenenii* leaf ethanol extracts are provided in the Supplementary Information (Figures **S2** and **S3**). Full-scan total ion chromatograms (TIC) acquired in ESI(+) and ESI(−) modes for both species are also available in the Supplementary Information (Figures **S4**–**S7**).

Results and discussion

Chemical components in Aloe species by UHPLC-PDA-MS/MS

The putatively annotated compounds (**1**–**7**) in *A. koenenii* and *A. vera* leaf epidermis were glycosides of anthrones and chromones (Table 1). Homonataloside B (**2**), 7-hydroxyaloin A or B (**4**) (anthrones), and isoaloeresin D or aloeresin D (**5**) (chromone) were annotated only in *A. vera* (Table 1). The annotation of the compounds was based on the MS/MS and UV spectral similarity to literature data, as well as their elution order in reversed-phase UHPLC (Lee et al. 2012; Wu et al. 2013; Zhong et al. 2015; Fan et al. 2018; Aldayel et al. 2020; Bendjedida et al. 2021).

Compound **1** (RT = 3.68 min) was detected in *A. koenenii* and *A. vera* extracts (Table 1). ESI(−)-MS spectrum of **1** (Figure **S8**) displayed a peak of *m/z* 393 $[M - H]^-$, which was attributed to deprotonated aloesin ($C_{19}H_{21}O_9^-$). The product ions of *m/z* 303, 273 and 231 (Table 1) are resulting of the losses of $C_3H_6O_3$ (90 u), two $C_2H_4O_2$ (120 u), and $C_6H_{10}O_5$ (162 u) molecules from the precursor ion (*m/z* 393), respectively (Scheme **S1**), so confirming the presence of the glycosyl moiety of the structure of aloesin. This chromene has also been previously identified in *A. barbadensis* (Wu et al. 2013, Fan et al. 2018), A. *vera* var. *chinensis*, *A. ferox* (C), and *A. arborescens* (Fan et al. 2018).

Compound **2** (RT = 6.89 min) was identified only in the *A. vera* extract (Table 1). ESI(−)-MS spectrum of **2** (Figure **S9**) displays a peak at *m/z* 593 $[M - H]^-$, which was annotated to be corresponding to the deprotonated homonataloside B ($C_{28}H_{33}O_{14}^-$). The product ions of *m/z* 473 (concerted loss of two $C_2H_4O_2$ molecules, or 120 u) and 431 (neutral loss of the glycosyl moiety) are consistent with the presence of a glycosyl moiety in its structure from the precursor ion (*m/z* 593), so as *m/z* 341, 297, 269, and 282, according to Scheme **S2**. Although in this study compound **2** was detected only

in the extract of *A. vera* (Table 1), its occurrence in other *Aloe* species was described in literature (Wu et al. 2013; Breaud et al. 2023).

Compound **3** (RT = 8.40 min) was detected in *A. koenenii* and *A. vera* extracts. ESI(−)-MS spectrum of **3** (Figure **S10**) presented the deprotonated molecule at *m/z* 447 $[M - H]^-$, which was tentatively attributed to 7-hydroxy-8-*O*-methylaloin ($C_{22}H_{23}O_{10}^-$). The precursor ion generated product ions of *m/z* 327 and 284 due to losses of 120 Da ($C_4H_8O_4$) and 163 Da ($C_6H_{11}O_5^•$) from the precursor ion of *m/z* 447, respectively (Scheme **S3**). These fragmentations are consistent with the presence of a glycosyl moiety in the structure of **3** (Lee et al. 2012).

Regarding compound **4**, the deprotonated molecule of *m/z* 433 $[M - H]^-$ (RT = 9.10 min) was detected only in *A. vera* extract (Table 1) and annotated as 7-hydroxyaloin A or B ($C_{21}H_{21}O_{10}^-$). ESI(−)-MS/MS spectrum of **4** (Figure **S11**) showed product ions of *m/z* 313 (loss of $C_4H_8O_4$, 120 Da) and *m/z* 270 (radical loss of a hexose, 163 Da) (Scheme **S4**), which confirms the presence of a glycosyl moiety in the structure of **4** (Lee et al. 2012).

Compound **5** was verified only in *A. vera* (Table 1). ESI(+)-MS spectrum of **5** (Figure **S12**) presented a peak of *m/z* 557 $[M + H]^+$ (RT = 11.95 min), which was attributed to the protonated molecule of isoaloeresin D or aloeresin D ($C_{29}H_{33}O_{11}^+$). The product ions of *m/z* 513 (loss of C_2H_4O, 44 Da), *m/z* 437 (loss of $C_4H_8O_4$, 120 Da), and *m/z* 393 (loss of the cumaroyl unit, 164 Da) confirmed the presence of the glycosyl and coumaroyl moieties, as shown in Scheme **S5** (Bendjedida et al. 2021).

Compounds **6** and **7** (RT = 14.13 and 14.56 min, respectively) were detected in *A. koenenii* and *A. vera* extracts (Table 1). ESI(+)-MS spectrum of **6** and **7** (Figure **S13** and **S14**) displayed the peak corresponding to their protonated molecules at *m/z* 419, which matches the empirical formula $C_{21}H_{23}O_9^+$. Thus, compounds **6** and **7** were putatively annotated as the epimers aloin B and aloin A, respectively, which were assigned based on the elution sequence (reversed phase) in the literature data (Aldayel et al. 2020). The product ions of *m/z* 401 (loss of H_2O, 18 Da), *m/z* 257 (loss of hexose, 162 Da), and the base peak of *m/z* 239 (loss of H_2O + hexose, 180 Da) (Schemes **S6** and **S7**) followed the results published by Aldayel et al. (2020).

The compounds annotated in *A. koenenii* have previously demonstrated several pharmacological activities. Aloin isomers (aloin A and aloin B) presented anti-inflammatory, anticancer, antimicrobial, antiviral, antiparasitic, anti-tumor, antioxidant, organ-protective, and anti-osteoporotic activities (Xiao et al. 2022; Yang et al. 2022). Aloesin demonstrated antidiabetic, antioxidant, and anti-inflammatory activities (Hameed et al. 2024).

Table 1
Annotated compounds in the leaf epidermis of *A. koenenii* and *A. vera* by UHPLC-PDA-MS/MS.

Compound	RT (min)	Cationized ions	MS^1 (*m/z*)	MS^2 *m/z* (neutral losses)	UV λ_{max} (nm)	Annotation	*A. koenenii*	*A. vera*	References
1	3.68	$[M - H]^-$	393	273 (-120), 245 (-148), 231 (-162)	251, 298	aloesin	+	+	(Wu et al. 2013; Fan et al. 2018)
2	6.89	$[M - H]^-$	593	473 (-120), 431 (-162)	220, 269, 331	homonataloside B	-	+	(Lee et al. 2012; Breaud et al. 2023)
3	8.40	$[M - H]^-$	447	327 (-120), 284 (-164)	220, 295	7-hydroxy-8-*O*-methylaloin	+	+	(Lee et al. 2012)
4	9.10	$[M - H]^-$	433	313 (-120), 270 (-163)	269	7-hydroxyaloin A or B	-	+	(Lee et al. 2012)
5	11.95	$[M + H]^+$	557	513 (-44), 437 (-120), 393 (-164)	223, 299	isoaloeresin D or aloeresin D	-	+	(Bendjedida et al. 2021)
6	14.13	$[M + H]^+$	419	401 (-18), 257 (-162), 239 (-180)	220, 266, 295, 355	aloin B	+	+	(Aldayel et al. 2020)
7	14.56	$[M + H]^+$	419	401 (-18), 257 (-162), 239 (-180)	220, 295, 355	aloin A	+	+	(Aldayel et al. 2020)

(+) presence, (-) absence

Conclusions

UHPLC–PDA–MS/MS profiling of the leaf epidermis extract of *A. koenenii* enabled a direct comparison with *A. vera*. The two species displayed an overlapping chemical signature dominated by anthrone- and chromone-related constituents, with *A. vera* showing additional features under the conditions evaluated. Overall, the data support chemotaxonomic proximity within the genus and establish a practical set of marker signals that can be applied to quality control and authentication of *Aloe* leaf materials. This baseline chemical profile also creates a clear starting point for subsequent studies exploring minor constituents and their contribution to biological activity.

Declarations

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Competing interests

The authors declare that they have no competing interests.

Author Contributions

Sven Zalewski: Material preparation, data collection, analysis and manuscript draft writing, Flávio A. Carvalho: Data analysis and manuscript draft writing, Luis V. S. Sacramento: Botanical identification, Caio H. Perego: Material preparation, Eduardo J. Crevelin & Antônio E. M. Crotti: Data collection, analysis and manuscript draft writing, André G. Santos: Critical analysis of manuscript.

Data Availability Statement

All data supporting the findings of this study are included in the article and its Supplementary Information file

Ethics approval

Not applicable. This study did not involve human participants or animals

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Figures

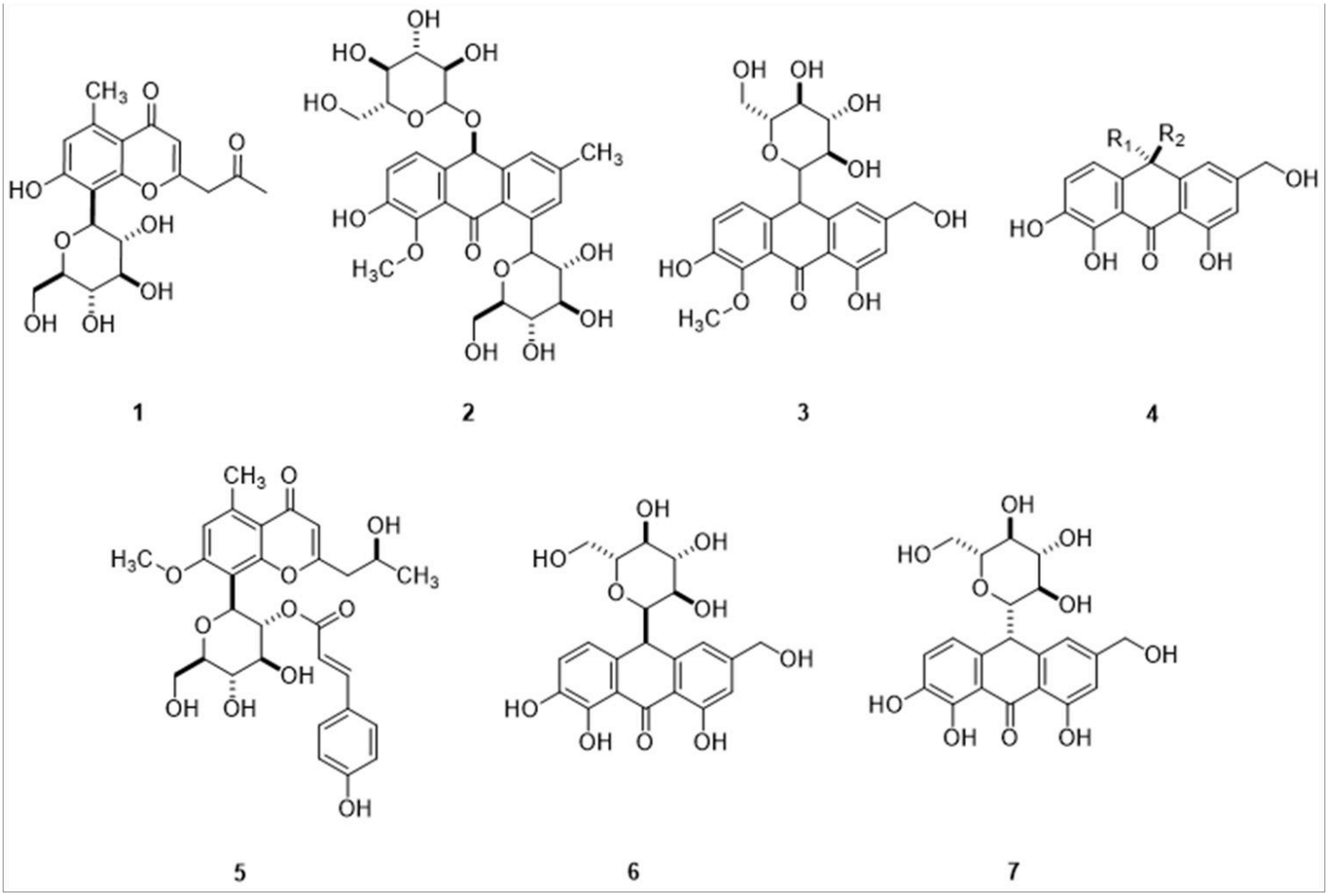

Figure 1

Unnumbered image in the Results and discussion section.

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