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Short Communication Article

Secondary metabolites from *Araucaria araucana* (Molina) K.Koch leaves

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ABSTRACT

The first phytochemical analysis concerning the non-volatile secondary components of one European specimen of *Araucaria araucana* (Molina) K.Koch led to the identification of five compounds: sandaracopimaric acid (**1**), ladanein (**2**), shikimic acid (**3**), 4-*O*-coumaroyl-quinic acid (**4**) and 5-*O*-coumaroyl-quinic acid (**5**). Compounds **2, 3** were reported for the first time in the species during this study.

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1. Introduction

Araucaria araucana (Molina) K.Koch is a coniferous tree belonging to the Araucariaceae family, native to Central Chile and Central-Western Argentina (https://powo.science.kew.org/). It presents harsh and thick leaves, 3-4 cm long and 1-3 large at the base, with a triangular shape and a cutting edge which remain on the tree for 10-15 years (Díaz-Vaz, 1984).

It widely grows in temperate climates of different areas worldwide where it has been introduced for which it is considered as the most rustic species of the genus (Díaz-Vaz, 1984).

This species is mainly known for its wood which is highly employed in several manufactures (Díaz-Vaz, 1984) but also for its resin which has been externally used in Chile and Venezuela to treat contusions, ulcers and to favor cicatrization (Schmeda-Hirshmann et al., 2005a).

A. araucana has been already studied for its phytochemical composition. Yet, these studies have only focused on specific classes of natural compounds and on non-European specimens except one. In particular, essential oil components have been reported from the leaves collected in Germany (Pietsch and König, 2000) and Australia (Briggs and White, 1975); biflavonoids have been identified from the leaves collected in India (Parveen et al., 1987); alkanes have been isolated from the leaf surfaces of different populations from the Andes (Rafii and Dodd, 1998); labdanes have been shown from different resins collected in Chile (Garbarino et al. 1987; Schmeda-Hirshmann et al., 2005a, 2005b); lignans have been evidenced in the heartwood collected in Chile (Céspedes et al., 2006) as well as in the stemwood, branchwood and knotwood (Bravo-Arrepol et al., 2020, 2023); different phenolic compounds have been found from the kernels collected in Chile (Schmeda-Hirshmann et al., 2021); several primary metabolites have been recognized in the nuts collected in Australia (Nadolny et al., 2023).

In this work, the first phytochemical analysis concerning the non-volatile metabolites of one European specimen of

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this species was reported. Its main aims were to expand the phytochemical knowledge and to start a chemophenetic evaluation on this species.

2. Experimental

2.1. Plant Material

Leaves of *A. araucana* (Fig. 1) (5.0 g) were collected in the Botanical Garden of Rome (geographical coordinates 41°53′32″ N, 12◦27′57″ E) in July 2021. The botanical identification was directly performed by a botanist of the garden and one of the authors (Dr. Claudio Scintu) by comparison of their morphological feature with those reported in the literature (Díaz-Vaz, 1984). A sample of this collection is kept in our laboratory for further reference under the voucher code AA13072021.

Fig. 1. Image of *Araucaria araucana* (Molina) K.Koch leaves.

2.2. Reagents and materials

The following materials and solvents were utilized during this study: ethanol 96% for the extraction procedure; *n*-butanol, distilled water and methanol as pure solvents or in a mixture among them all as eluting systems for the column chromatography separation on silica gel (40-63 µm) used as stationary phase; sulfuric acid (2.0 N) for the developments of the TLCs; deuterated solvents ($CDCl_3$ and D_2O) for the identification of metabolites by means of NMR spectroscopy; HPLC-grade methanol for the identification of metabolites by means of mass spectrometry. All the solvents having RPE purity grade, if not differently specified, together with the deuterated solvents, the TLCs and the HPLC-grade methanol were purchased from Merck (St. Louis, Missouri, USA) whereas the silica gel was purchased from Fluka Analytical (Bergamo, Italy).

2.3. Instrumentation

^{1}H NMR spectra were recorded at 298 K on a Jeol JNM-ECZ 600R spectrometer with a magnet operating at 14.09 T corresponding to a proton resonance frequency of 600.19 MHz and equipped with a Jeol multinuclear *z*-gradient inverse probehead, acquired with 32 transients, a spectral width of 9013.7 Hz (corresponding to 15 ppm) and 64K data points for an acquisition time of 7.3 s, with a recycle delay set to 7.7 s. The chemical shifts were referenced to TMS (s, 0 ppm) for spectra in $CDCl_3$, while the HDO signal (s, 4.79 ppm) was set as reference for spectra in D_2O.
MS spectra were acquired on a triple quadrupole mass spectrometer PE-Sciex API-3000® (Perkin Elmer Sciex, Toronto, ON, Canada), equipped with an ESI source operating in the negative and/or positive ion mode, in a mass spectral range of 100-1000 *m/z*, with the capillary ion voltage set at 5000 V for the positive ionization and -4500 V for the negative one, with high-purity nitrogen used as a curtain gas (5 L/min) and air as nebulizer (2 L/min) and drying gas (30 psi), with the temperature to heat the drying gas set at 100 °C, with a flow rate of sample infusion of 20 µL/min, with 20 acquisitions per sample, with the full width at half maximum (FWHM) set at *m/z* 0.7 ± 0.1 in each mass-resolving quadrupole to operate with a unit resolution, with data acquired and elaborated by Analyst® 1.6 software (AB Sciex, Washington, USA).

2.4. Extraction and separation procedure

A portion of the collected leaves (4.9 g) was extracted by maceration in ethanol 96% (500 mL) three times for 14 days, each. The extracting solvent was evaporated at reduced pressure at 50 °C. Throughout the concentration procedure, pH was checked on litmus paper to verify that pH was not too acid or basic (meaning between the range 5.5-8.5) given that an extreme acidity or alkalinity might cause secondary reactions in the extract such as the hydrolysis of ester and glycosidic bonds. In this case, pH was 8. The obtained dried green extract weighed 1.8 g.
An aliquot of this extract (1.5 g) was subjected to a chromatographic separation on silica gel (45 g, ratio 1:30 *w/w*). The eluting system was a mixture of *n*-butanol and distilled water at the concentration ratio of 82:18 *v/v* (400 mL). During the chromatographic run, the polarity of the eluting system was raised in order the let the elution of more polar compounds by passing to a mixture of *n*-butanol, methanol, and distilled water at the concentration ratio of 70:10:30 *v/v/v* (200 mL). From this chromatographic separation, all the compounds were identified by comparison of their spectroscopic and spectrometric data with those as reported in the literature: sandaracopimaric acid (**1**) (Venditti et al., 2017) in mixture with ladanein (**2**) (Frezza et al., 2022) and lipids (ratio not calculable) from the assembly of fractions 4-20 (254.5 mg); shikimic acid (**3**) (Frezza et al., 2022) in mixture with saccharides (ratio 5:1 *w/w*) from the assembly of fractions 26-51 for the total weight of 35.9 mg; 4-*O*-coumaroyl-quinic acid (**4**) and 5-*O*-coumaroyl-quinic acid (**5**) (Frezza et al., 2022) in mixtures with saccharides (ratio not calculable) from the methanol column wash (47.8 mg).

2.5. NMR data of the identified compounds

Sandaracopimaric acid (**1**): ^{1}H NMR (600 MHz, $CDCl_3$): 5.76 (1H, dd, *J* = 17.4/10.6 Hz, H-15), 5.21 (1H, s, H-14), 4.91 (1H, dd, *J* = 17.4/1.5 Hz, Ha-16), 4.87-4.85 (1H, m, Hb-16), 2.35-2.29 (1H, m, Ha-7), 2.18-2.12 (1H, overlapped, Hb-7), 1.20 (3H, s, H-19), 1.03 (3H, s, H-17), 0.83 (3H, s, H-20).
ESI-MS: *m/z* 325.11 $[M+Na]^+$; *m/z* 301.04 $[M-H]^-$.
Ladanein (**2**): ^{1}H NMR (600 MHz, $CDCl_3$): 7.42 (2H, d, *J* = 9.1 Hz, H-2′ and H-6′), 6.86 (2H, d, *J* = 9.1 Hz, H-3′ and H-5′), 6.60 (1H, s, H-3), 6.58 (1H, s, H-8), 3.82 (3H, s, 4′-OMe), 3.79 (1H, s, 7-OMe).
ESI-MS: *m/z* 325.11 $[M+Na]^+$; *m/z* 301.04 $[M-H]^-$.
Shikimic acid (**3**): ^{1}H NMR (600 MHz, D_2O): 6.46-6.43 (1H, m, H-2), 4.42 (1H, t, *J* = 4.5 Hz, H-3), 4.02-3.98 (1H, m, H-5), 3.72 (1H, dd, *J* = 9.2/4.3 Hz, H-4), 2.78 (1H, dd, *J* = 17.9, 5.5 Hz, H-6a), 2.21 (1H, dd, *J* = 17.9, 7.0 Hz, H-6b).
ESI-MS: *m/z* 173.22 $[M-H]^-$.
4-*O*-coumaroyl-quinic acid (**4**): ^{1}H NMR (600 MHz, D_2O) δ: 7.78 (1H, d, *J* = 16.1 Hz, H-β), 7.60 (2H, d, *J* = 8.4 Hz, H-2′, H-6′), 6.96 (2H, d, *J* = 8.4 Hz, H-3′, H-5′), 6.49 (1H, d, *J* =

16.1 Hz, H-α), 4.30-4.26 (2H, overlapped signals, H-3, H-5), 2.27-2.05 (4H, overlapped signals, H-2, H-6).
ESI-MS: *m/z* 337.34 [M-H]$^-$.
5-*O*-coumaroyl-quinic acid (**5**): ^{1}H NMR (600 MHz, D_2O) δ: 7.73 (1H, d, *J* = 15.9 Hz, H-β), 7.52 (2H, d, *J* = 8.6 Hz, H-2′, H-6′), 6.88 (2H, d, *J* = 8.6 Hz, H-3′, H-5′), 6.44 (1H, d, *J* = 15.9 Hz, H-α), 4.30-4.26 (2H, overlapped signals, H-3, H-5), 2.27-2.05 (4H, overlapped signals, H-2, H-6).
ESI-MS: *m/z* 337.34 [M-H]$^-$.

3. Results and discussion

The first phytochemical analysis concerning the non-volatile secondary metabolites of the leaves of one *A. araucana* specimen collected in Europe led to the identification of five compounds, namely sandaracopimaric acid (**1**), ladanein (**2**), shikimic acid (**3**), 4-*O*-coumaroyl-quinic acid (**4**) and 5-*O*-coumaroyl-quinic acid (**5**) (Fig. 2).

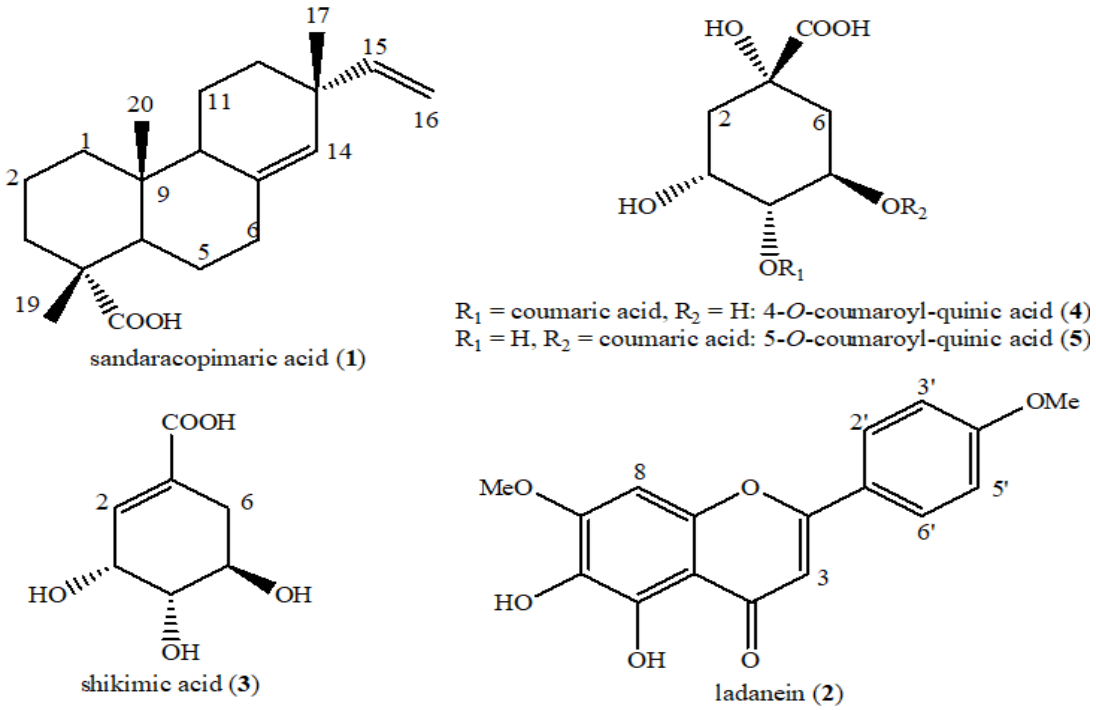

Fig. 2. Structures of the identified compounds in *A. araucana* leaves.

These compounds belong to three different classes of natural compounds *i.e.*, diterpenoids (**1**), flavonoids (**2**), organic acids (**3-5**).
To the best of our knowledge, ladanein (**2**) and shikimic acid (**3**) were identified in the species for the first time during this study. In fact, their presence has been previously reported only from the leaves of *Araucaria columnaris* (G. Forst.) Hook (Frezza et al., 2022) and *Araucaria cunninghamii* Mudie (Frezza et al., 2024). Conversely, sandaracopimaric acid (**1**) has been already found in the resin of this species (Schmeda-Hirshmann et al., 2005a) while 4-*O*-coumaroyl-quinic acid (**4**) and 5-*O*-coumaroyl-quinic acid (**5**) have been previously reported from its kernels (Schmeda-Hirshmann et al., 2021).

4. Concluding remarks

The first phytochemical analysis on the non-volatile secondary metabolite content of the leaves of one *A. araucana* specimen collected in Europe, and, in particular, in the Botanical Garden of Rome, allowed the identification of five compounds, two of which were reported in the species for the first time. The presence of the newly reported compounds widens the phytochemical knowledge of this species whereas the presence of the already reported compounds provides further evidence on their high occurrence in the species.

Authors contribution

CF and LF performed the separation procedure and elucidated the chemical structures; OG and FS performed the NMR experiments; IS performed the MS experiments; CS collected and provided the plant material; DDV and FA supervised the entire work. All the authors wrote and edited the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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