

Phenolic Compounds from Leaves of *Cariniana estrellensis* (Raddi) Kuntze (Lecythidaceae): A Brazilian Atlantic Forest Tree

Angelica N. Tiepo,^{1b}*^{#,a} Isabel D. Coutinho,^{#,b} Guilherme O. Machado,^b
Halley C. Oliveira,^{1b}^a José Antonio Pimenta,^{1b}^a Liliane Marcia M. Henning,^{1b}^c
Luiz Alberto Colnago^b and Renata Stolf-Moreira^{1b}^{*,a}

^aDepartamento de Biologia Animal e Vegetal, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid - PR445, km 380, Campus Universitário, 86057-970 Londrina-PR, Brazil

^bEmbrapa Instrumentação, Rua XV de Novembro, 1452, 13560-970 São Carlos-SP, Brazil

^cEmbrapa Soja, Rodovia Carlos João Strass, s/n, 86085-981 Londrina-PR, Brazil

Cariniana estrellensis (Raddi) Kuntze seedlings have been used in forest restoration programs in the Atlantic Forest biome. However, despite the economic and ecological relevance of this species, to date no general method for rapid identification of primary and secondary metabolites of *C. estrellensis* leaves has been reported. In this work, we explore the feasibility of using rapid analysis by nuclear magnetic resonance techniques and liquid chromatography coupled to diode array, charged aerosol, and mass detection. The main secondary metabolites identified were hydrolysable tannins, quinic acid, hydroxycinnamic acids (*trans-p*-coumaric acid, *cis-p*-coumaric acid, and *trans*-ferulic acid), and flavonoids (kaempferol and quercetin derivatives). These compounds are particularly useful as chemotaxonomic markers for the genus *Cariniana* and for the family Lecythidaceae, and may also be important for pharmacological uses and for the survival of the seedlings in reforestation programs.

Keywords: tannin, flavonoids, kaempferol, quercetin, quinic acid, reforestation

Introduction

The Brazilian Atlantic Forest is one of the 25 global biodiversity hotspots and comprises the second biggest tropical rain forest on the American continent. In the last decade, deforestation rates have increased in Brazilian forests, which negatively affects the environmental homeostasis and boosts the rates of local species extinction.^{1,2} These aspects enhance the need for restoration in these sites, which is essential for the maintenance of flora and fauna biodiversity.

The Lecythidaceae family is spread across Neotropical sites, and includes the genus *Cariniana* Casar., which comprises nine species.^{3,4} Non-pioneer trees, such as *Cariniana*, have a special role in epiphytes survival, carbon sequestration and accumulation, and input of soil organic matter through root exudation and litterfall.⁵⁻⁸

The wood of *Cariniana* is widely used for manufacturing furniture, casks in the cachaça ageing process and bark in folk

medicine.^{9,10} Infusion of *Cariniana decandra* Ducke (1925) bark is recommended for cancer as well as high fever and skin infections.^{11,12} Bark from *Cariniana rubra* Gardner ex Miers (1874) is recommended for kidneys, while maceration and decoction of *Cariniana estrellensis* (Raddi) Kuntze is used in cases of general infection, depurative, and ulcer.^{4,13} *Cariniana brasiliensis* Casar. presented *in vitro* 90% of inhibition of mushroom tyrosinase and antifungal activities were reported for bark from *C. rubra* Gardner ex Miers.^{14,15}

Moreover, non-pioneer trees used in Brazilian reforestation programs, such as *Cariniana estrellensis* (Raddi) Kuntze contribute to high species richness which may allow biodiversity conservation.^{6,7,16,17} The presence of *C. estrellensis* in the Atlantic Forest is essential for maintenance of the environmental homeostasis, since its flowers are pollinated by bees and its seeds are taken up by fauna species, such as monkeys.¹⁸⁻²⁰

In addition, the use of native trees in reforestation programs can supply the demand for native species wood, reducing predatory exploration, as *C. estrellensis* wood is intensively explored by industry, as well as increasing the economic potential by using phytochemicals, through

*e-mail: angelicantiepo@gmail.com; stolfrenata@gmail.com

[#]Angelica N. Tiepo and Isabel D. Coutinho contributed equally for the development of this study, that is why the first authorship is shared.

Editor handled this article: Paulo Cezar Vieira

bioactive compounds, in the cosmetic, pharmaceutical and food industry.²¹

Although traditional uses of *Cariniana* species are described, there are few reports about the chemical composition of the leaves and bark of species of this genus. In *Cariniana domestica* (Mart.) Miers lupeol, amyirin, sitosterol, stigmasterol, gallic acid, chlorogenic acid, rutin, quercetin and kaempferol were identified in bark extracts.^{22,23} For *C. estrellensis*, it has been reported that the plant growth-promoting bacteria association induced an increase in chlorogenic acid, gallic acid, rutin and synapic acid in leaves of drought-stressed seedlings.¹⁶

Despite its economic and ecological relevance, and the ethnobotanical studies on the *Cariniana* species, a general method and study for rapid identification of primary and secondary metabolites of *C. estrellensis* leaves has never been reported for metabolomic studies. A prerequisite for chemotaxonomy, ecological and metabolomic studies is to know the qualitative and quantitative composition as well as the occurrence of metabolite groups in the evaluated plant species. Nuclear magnetic resonance (NMR) is a very useful proton experiment in metabolomic analysis due to its important structural and quantitative information. NMR and chromatography methods combined with mass spectrometry and ultraviolet spectroscopy are the most important high-throughput analytical techniques for metabolomics screening. Thus, in the current paper we applied different chromatographic systems and NMR techniques to provide the first comprehensive data on phenolic compounds for *Cariniana estrellensis* leaves, which may be useful to support future ecological and metabolomic studies.

Experimental

Plant material

Cariniana estrellensis (Raddi) Kuntze (Lecythidaceae) is a native species from seasonal semi-deciduous forests (a phytophysognomy of the Brazilian Atlantic Forest) and is classified as a shade-tolerant or non-pioneer species.

The seeds used in the present study were collected in forest fragments in northern Paraná, southern Brazil, the number of the registration in SisGen is AB50EA6 and the herbarium registration is *Cariniana estrellensis* (Raddi) Kuntze, determiner J. E. L. S. Ribeiro (24-March-2022), FUEL 56.481. The seedlings were grown under ideal conditions of light, water, and nutrients. They were maintained in plastic bags (2 L, 15 cm high and 13 cm diameter) containing a mixture (1:1) of the inert substrate and fertile soil (pH 5.8; cation exchange capacity 4.4 cmolc dm) characterized as clayey oxisol. Five months

after germination, the third completely expanded leaf was collected from ten different seedlings. In the present study, these ten leaves were analyzed in triplicate.

Sample preparation

Deuterium water (D₂O), deuterated methanol (CD₃OD), ethanol (HPLC) and sodium salt of trimethylsilylpropionic acid (TSP-*d*₄) were purchased from Sigma-Aldrich (St. Louis, USA).

The extracts were obtained from 30 mg of milled dry leaves in 1 mL of CD₃OD:D₂O (8:2 v/v). The milled dry leaves and 1 mL of mixture of deuterated solvent were mixed in a vortex for one minute. Next, the contents of the tube were heated at 50 °C in a water bath for 10 min. The samples were then centrifuged for 5 min at 4 °C. The supernatant was kept at 4 °C overnight, centrifuged, and 500 µL of supernatant was transferred to a tube. Subsequently, 10 µL of sodium salt of trimethylsilylpropionic acid (TSP-*d*₄, 1.00 mol L⁻¹) were added to each extract and transferred to a 5 mm NMR tube. For chromatographic analysis the extract was prepared in the same way as for NMR, except we used an ultrasonic bath at 50 °C for 10 min instead of a water bath. Finally, the extract was centrifuged and 500 µL of supernatant was transferred to a vial.

NMR and liquid chromatography coupled to diode array detection tandem mass spectrometry (LC-DAD-MS/MS) instrumentation

C. estrellensis leaf hydroalcoholic extract was analyzed by 1D and 2D NMR experiments. The spectrum was acquired at 300 K with an Avance III HD spectrometer operating at 600 MHz and equipped with a 5-mm BBO probe (Bruker, Germany). ¹H spectra were acquired using nuclear Overhauser effect spectroscopy (NOESY) 1D with a 2.00 s presaturation delay and an acquisition time of 2.69 s (64 k points), an accumulation of 256 transients, and a spectral width of 15 ppm. All free induction decay (FIDs) were automatically Fourier transformed after the application of an exponential window function with a line broadening of 0.3 Hz. Phase and baseline corrections were carried out within the instrument software. ¹H NMR chemical shifts were referenced to TSP-*d*₄ at δ 0.00. Secondary metabolites were identified through 1D and 2D NMR spectra (correlation spectroscopy (COSY)), heteronuclear single quantum correlation (HSQC). In addition, ultra-performance liquid chromatography coupled to diode array detection and charged aerosol detection (UPLC-DAD-CAD) Thermo Scientific®, Dionex, (Sunnyvale, California, USA), and ultra-high-pressure

liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-DAD-QTOF-MS^E), (Waters[®], Massachusetts, USA) were employed to support metabolite identification.

The chromatographic method was developed using UHPLC (Ultimate3000, Dionex[®]) equipped with two pumps and, an automatic sampler, coupled to a diode array detector and Corona Veo charged detector. Separation was carried out with a Kinetex[®] C-18 (50 × 2.1 mm × 2.6 μm) and column temperature was maintained at 40 °C. Elution was conducted in a linear gradient program containing a 0.1% formic acid - water (A) and 0.1% formic acid - ethanol (B) solvent system; gradient: 2 to 30% of B, 0-20 min, 20 to 100% of B, 5 min and maintained at 100% of B for 5 min, at a flow rate of 0.5 mL min⁻¹. 10 μL of sample was injected for analysis. The UV-DAD detector was set to the wavelength range of 200-600 nm and the chromatograms were plotted at 254, 330, and 350 nm. A CAD response was set at 100 pA, a high filter was selected, and air regulated at 35 psi was introduced to the detector. Firstly, eluate was introduced into a DAD and then directly to the CAD instrument. Data processing was performed with Chromeleon 6.8 (Dionex). For metabolic annotation, *Cariniana estrellensis* leaf hydroethanolic extract was analyzed by UPLC-DAD-QTOF-MS^E (Waters Acquity[®] H-Class pump; Acquity[®] FTN autosampler; Acquity[®] PDA detector and Xevo G2-XS QToF). The separation conditions were the same as for UPLC-DAD-CAD analysis. Mass spectra were acquired in negative and positive modes over a *m/z* range of 100-1000, in separated runs. The parameters for electrospray ionization (ESI) source were: capillary voltage 3.0 kV, cone voltage 30 V, source temperature 100 °C, and cone gas flow 50 L h⁻¹. Desolvation temperature was 650 °C with a desolvation gas flow of 800 L h⁻¹. The MS^E data were acquired in centroid mode using a scan range of 100-1000, scan time of 0.1 s, lower energy 20 eV, and a higher collision energy ramp 20-50 eV. Data processing was carried out with MassLynx 4.1 software (Waters). Metabolite annotation was performed using UNIFI[®] Scientific Information System, version 1.6, Waters[®].

Results and Discussion

Phytochemical fingerprinting of *Cariniana estrellensis* leaf hydroethanolic extract was obtained using NMR, UPLC-DAD-CAD and UPLC-DAD-QTOF-MS^E. The arrangement of chemical shift and coupling constant by ¹H NMR, retention time, UV, and CAD detection by UPLC-DAD-CAD, mass spectrum (*m/z*, negative mode), mass fragments (*m/z*) by UPLC-QTOF-MS^E combined with the UNIFI platform and minimal sample preparation enabled reliable metabolite annotation and a rapid procedure

for the screening of *C. estrellensis* seedlings. Using a database available in UNIFI software, 442 compounds were matched and only peaks with an intensity greater than 5% were selected manually for metabolite annotation.

UPLC-DAD-CAD analysis through UV data showed the presence of three classes of phenolic compounds identified at 350 nm (flavonols), 320 nm (hydroxycinnamic acid), and 270 nm (hydrolysable tannins) (Figure 1) and UV spectra compared to literature.²⁴ Annotation of hydrolysable tannins was based on mass spectra and ion fragments provided by UPLC-QTOF-MS^E analysis combined with a database available on the UNIFI platform (Table 1). Their fragmentation patterns were in accordance with those described in the literature for the annotated metabolites. Thus, the losses of 152 and *m/z* 169 correspond to galloyl groups, while *m/z* 301 indicates the presence of an ellagitannin group.²⁵ The metabolite eluted in 4.7 and 5.2 min in UPLC-QTOF-MS^E was annotated as castalagin and shows *m/z* 933.0650 and ion fragments 765 (loss of galloyl group), 301 (hexahydroxydiphenoyl (HHDP)) and 613 (HHDP-glu). Interestingly, charged aerosol detection showed that castalagin derivatives eluted at 6.0 and 6.4 min in the UPLC-DAD-CAD system are major secondary metabolites present in hydroethanolic extract of *C. estrellensis*. CAD is a universal detector and responds to all nonvolatile species and it was used as an orthogonal detector to provide more comprehensive analysis of secondary metabolism of *C. estrellensis*.²⁷

Quinic acid was identified by the multiplet δ 1.90 m in the ¹H NMR and *m/z* 191.0558 at 0.29 min by UPLC-DAD-QTOF-MS^E. Signals from *trans-p*-coumaric acid (δ 7.72 d, 15.99 Hz; δ 6.46 d, 16.04 Hz), *cis-p*-coumaric acid (δ 5.97 d, 12.54 Hz; δ 7.03 d, 12.91 Hz), and *trans-ferulic acid* (δ 6.48 d, 16.03 Hz; δ 7.70 d, 15.94 Hz) were assigned in ¹H NMR spectra and correlations between olefinic hydrogens with *Z* configuration (*J* 16 Hz) and *E* configuration (*J* 12 Hz) were confirmed in the COSY ¹H-¹H spectra through correlations between proton at δ 7.72 with δ 6.46 d corresponding to H2 and H3 of *trans-p*-coumaric acid and δ 5.97 with δ 7.03 corresponding to H2 and H3 *cis-p*-coumaric acid. The presence of coumaric acid was confirmed due to *m/z* 163.0398 and UV spectrum λ_{max} 311, while the *m/z* 193.0499 corresponded to ferulic acid with UV spectrum λ_{max} 324 and confirmed with the literature.²⁸

Kaempferol and quercetin derivatives were identified by their UV spectra and fragmentation patterns and only major flavonols had characteristic signals assigned in ¹H NMR spectra. Kaempferol-di-*O*-glucoside was identified mainly by the doublet in 8.05 ppm due to the coupling of H-3',4' and the overlapping doublets δ 6.99 referring to H-2',6', confirming the presence of an AA'BB', and the signals δ 6.92

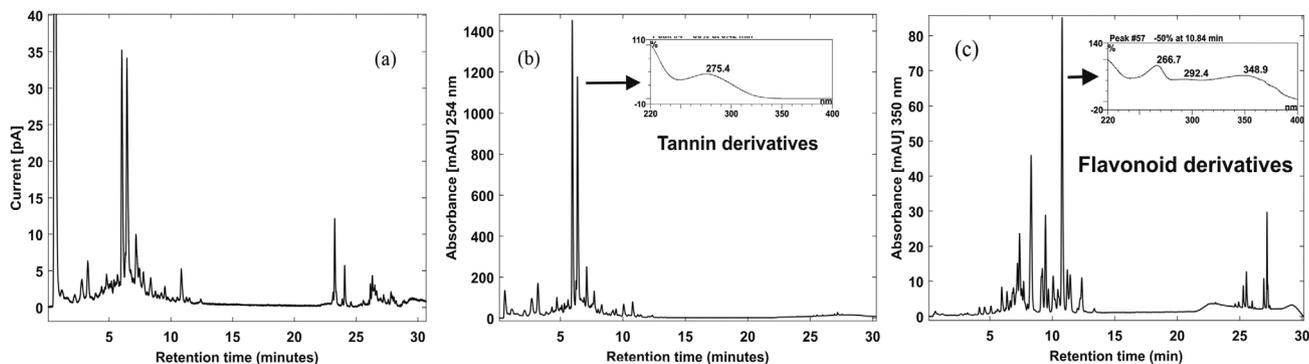


Figure 1. UPLC-DAD-CAD chromatogram from *Cariniana estrellensis* leaf hydroalcoholic extract. (a) CAD chromatogram; (b) UV-Vis chromatogram at 254 nm; (c) UV-Vis chromatogram at 350 nm.

Table 1. Metabolites identified by UPLC-DAD-QTOF-MS^E in the *Cariniana estrellensis* leaf extract

t_R / min	Annotated metabolite	Observed m/z [M – H] [–]	Mass error / Da	Fragment ions m/z	UV
0.29	quinic acid ^a	191.0558	0.3	–	–
0.63	digalloyl hexoside isomer I	483.0775	0.6	331.0659; 169.0134	–
0.91	pedunculagin	783.0687	0.1	481.0607, 300.99782, 275.01850	–
1.29	digalloyl hexoside isomer II	483.0770	0.6	331.0655, 169.0131	268/353 ^b
2.34	geraniin	951.0753	0.8	907.0839, 783.0659, 300.9977	–
2.42	mallotinic acid isomer I	785.0842	0.1	633.07157, 483.07586, 419.0598, 249.0393, 169.0133	272 ^c
3.73	euphormisin	635.0890	0.0	465.0656, 169.01334	272 ^c
3.89	mallotinic acid isomer II	785.0842	0.1	633.07157, 483.07586, 419.0598, 249.0393, 169.0133	272 ^c
4.55	trigalloyl hexoside	635.0892	0.2	483.0764, 313.0551, 169.01345	272
4.74	castalagin	933.0650	1.0	858.0654, 765.0568, 749.06136, 613.0450, 00.9982, 169.0134	272 ^c
5.18	potentillin	935.0798	0.2	917.0678, 765.0568, 300.9979, 169.0134	272 ^c
5.92	casuarinin	935.0785	1.2	765.0566, 300.9977, 169.0133	272 ^c
6.17	<i>trans-p</i> -coumaric acid ^a	163.0398	0.3	119.0498	311 ^d
6.36	<i>cis-p</i> -coumaric acid ^a	163.0398	0.3	119.0498	311 ^d
6.97	quercetin-di-glucoside ^a	625.1410	0.0	301.0354, 327.0489	–
7.01	kaempferol tri-glucoside	771.1993	0.4	591.1335, 465.0665, 327.0489, 285.0383, 227.0336	–
7.23	<i>trans</i> -ferulic acid ^a	193.0499	0.3	178.0261	324 ^d
7.24	<i>cis</i> -ferulic acid ^a	193.0503	0.3	178.0261	324 ^d
7.48	tetragalloyl hexoside	787.1002	0.3	617.0769, 465.0661, 295.0446, 169.0134	–
7.91	galloylhyperin	615.0993	0.1	463.0868, 300.02631, 169.01337	–
7.99	quercetin-di-glucoside ^a	625.1412	0.2	301.03253	262/358 ^b
8.24	kaempferol-di- <i>O</i> -glucoside ^a	609.1462	0.1	463.0864, 285.0315, 227.0337	264/348 ^b
8.48	quercetin-glucosyl-rhamnoside	609.1464	0.3	301.0333	262/358 ^b
8.85	quercetin glucoside	463.0874	0.8	300.9985	–
8.87	ellagic acid	300.9985	0.5	–	–
9.24	kaempferol-galloyl-glucoside	599.1041	–	447.0915, 313.0553, 285.0382, 169.0133	254/366 ^b
9.67	quercetin-arabinoside	433.0770	0.6	300.0263, 271.0236, 243.0286	–
9.58	kaempferol-di-glucoside	609.1463	0.2	285.03942	266/348 ^b
9.98	kaempferol-glucosyl-arabinoside	579.1358	0.3	284.0317	266/349 ^b
10.97	kaempferol glucoside	447.0926	0.7	285.0352	–
11.01	kaempferol arabinoside	417.0821	0.6	285.0352	–
11.17	kaempferol-arabinosyl-glucoside	593.1510	0.2	285.0388	267/346 ^b

^aData from ¹H NMR spectrum are described in text. ^bMabry *et al.*; ²⁴ ^cGarcía-Villalba *et al.*; ²⁵ ^dSchwarz *et al.*²⁶

and 7.02 corresponding to H-6 and H-8, which confirmed the flavonoid unit. Through the analysis by UPLC-DAD-QTOF-MS^E with database available in UNIFI, it was possible to identify that the kaempferol-di-*O*-glucoside eluted in 9.58 min, with the spectrum on the UV λ_{\max} 265/348 nm and m/z 609.1463 [M – H][–]. MS^E spectrum from the ion product showed ion fragments m/z 285 in the negative mode, characteristic of the *O*-flavonol pattern due to base peak corresponding to the aglycone product ion. The mass loss of 324 corresponds to two glucose units, which confirms the presence of two sugar moieties connected to kaempferol. The search in the database present in UNIFI allowed identification of six kaempferol derivatives; four which were not detected in the ¹H NMR spectra, but could be assigned by m/z spectra, fragment ion and UV spectra and are described in Table 1 and in the Figures 1c, 2 and S2a (Supplementary Information (SI) section), all the kaempferol and quercetin date were confirmed by the literature.²⁹

Quercetin-di-glucoside was identified in the ¹H NMR spectrum with signals characteristic of a tri-substituted system in ring C due to the double doublets δ 7.20 (8.62 and 1.96 Hz), and doublets δ 6.88 (8.63 Hz), and δ 7.32 (1.80 Hz), which correspond to H-6', 5' and 2', respectively. The H-6 and H-8 were detected as broad singlets at 6.94 and 7.07 ppm. The glycosidic unit assignment was also performed according to the UPLC-DAD-QTOF-MS^E, in which the quercetin di-glucoside eluted in 6.97 min, with UV spectrum λ_{\max} 262/358 nm and m/z 625.1410 [M – H][–]. Ion fragments of m/z 301 in the negative mode, which corresponds to the aglycone unit and the mass loss

of 324 corresponds to two glucose units, thus confirming the presence of a di-glycosylated quercetin derivative. Through the analysis by UPLC-DAD-QTOF-MS^E with the database available in UNIFI, four glycosylated derivatives of quercetin in low concentration were annotated, which were not detected in the ¹H NMR spectra, but are also described in Table 1, Figures 1c, 2 and S2b (SI section).

Glucose (δ 5.27 d, 3.70 Hz; δ 4.63 d, 7.92 Hz), lactate (δ 1.30 d), malic acid (δ 2.66 dd 3.1 and 15.1 Hz), succinic acid (δ 2.39 s), citric acid (2.54 d, 16.5 Hz) and hydroxybutyric acid (δ 1.35 s) were assigned by Chenomx NMR suite³⁰ (Figure S1, SI section), while only characteristic signals of secondary metabolites were extracted from ¹H and 2D NMR spectrum due to coalescence of the signals of mixture analysis. A set of multiplets 2.60, 2.70, 2.95, and 6.45 ppm could not be identified in the ¹H NMR spectrum. Three peaks detected in the total ion chromatogram could be not identified and were therefore described as unknown compounds (Table 1 and Figure S1, SI section).

Phytochemical investigations have been reported for species from the family Lecythidaceae, which indicated the presence of polyphenols, such as flavonoids (naringin, rutin, luteolin, and kaempferol derivatives) and cinnamic acid derivatives, alkaloids, and triterpenes in the leaves.^{10,31,32} In *C. estrellensis* the presence of trigonelline, chlorogenic acid, gallic acid, rutin, sinapic acid, epicatechin, and catechin was recently reported in drought-stressed seedlings.¹⁶ However, the current study is the first to demonstrate the presence of hydrolysable

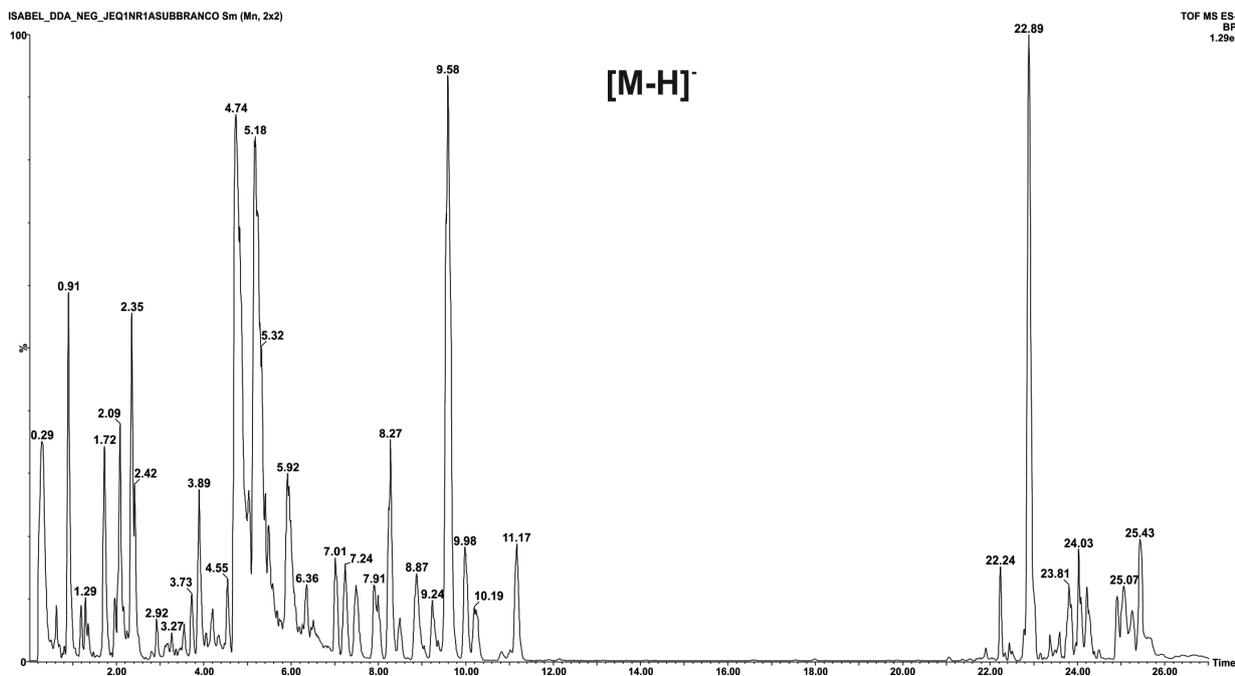


Figure 2. UPLC-DAD-MS/MS chromatogram from *Cariniana estrellensis* leaf hydroalcoholic extract in negative mode.

tannins in *C. estrellensis* leaf extracts, only ellagic acid had previously been identified in *Cariniana domestica* and in *Napoleona vogelii* (Lecythidaceae), being tannins related to the antioxidant and wound healing capacity.³³⁻³⁵

Secondary compounds have been considered a reliable taxonomic indicator for plants, and are an important tool in taxonomic elucidation.³⁶ Similar compounds to those identified in the present study have already been found in other genera from the Lecythidaceae family. For example, gallic acid, ferulic acid, and kaempferol were detected in leaves of *Barringtonia racemosa* (L.) Spreng., kaempferol 3-*O*-glucoside, quercetin 3-*O*-glucoside, kaempferol 3-*O*-rutinoside, and quercetin 3-*O*-rutinoside were found in leaves of *Barringtonia asiatica* (L.) Kurz; and quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside were identified in leaves of *Lecythis pisonis*.^{31,32,37}

Based on the above-mentioned metabolites, *O*-glycoside flavonols are chemotaxonomic markers of the Lecythidaceae family. Phenolic compounds stand out because they are widely distributed and have many ecological and pharmacological functions.¹⁵ The metabolites identified in the present study may have an important role for plant survival in reforestation programs, since the majority are polyphenols that are involved in tolerance to environmental stresses.³⁸ Many phenolic compounds have antioxidant properties and can be used to prevent free-radical-induced deleterious effects, as reported for leaf extracts from *Rosa canina* L., *Rosa rubiginosa* L. and *Alchemilla mollis* (Buser) Rothm.³⁹ Ferulic acid, *p*-coumaric acid, and kaempferol, which were also found in the present study, were related to antioxidant activity in *Origanum vulgare* L., *Lavandula angustifolia* Mill (1768) and *Melissa officinalis* L.⁴⁰

Conclusions

Our results show that leaves of *C. estrellensis* are rich in phenolic compounds with antioxidant properties already described in literature as well as a source of new compounds still not explored. In addition, the present study provided knowledge on the phytochemical profile of leaves from *C. estrellensis*; which is important as a chemotaxonomy trait, and in ecological and metabolomic studies. Furthermore, the metabolite screening indicated the presence of antioxidant compounds for *C. estrellensis* that have not previously been reported and may have importance in pharmacological uses and in plant survival.

Supplementary Information

Supplementary information (¹H NMR spectra and

LC-MS-DAD chromatogram) from *Cariniana estrellensis* leaf hydroalcoholic extract are available free of charge at <http://jbcbs.sbj.org.br> as PDF file.

Acknowledgments

The authors thank the Laboratory of Biodiversity and Restoration of Ecosystems at the State University of Londrina for making the seeds available.

The assistance provided by the NuBBE group of São Paulo State University (UNESP) for the LC-DAD-CAD and UPLC-DAD-QTOF-MS^E measurements is also acknowledged.

The assistance provided by the Embrapa Instrumentação and Embrapa Soja for the NMR measurements is also acknowledged.

The authors thank to the Superintendence of Science, Technology and Higher Education (SETI), Araucaria Foundation and to the State University of Londrina (PROPPG) for a piece of the financial support.

Author Contributions

Angélica N. Tiepo was responsible for conceptualization, formal analysis, investigation, supervision, validation, visualization, writing original draft, and writing review and editing; Isabel D. Coutinho for conceptualization, formal analysis, investigation, methodology, visualization, and writing original draft, review and editing; Guilherme O. Machado for formal analysis, investigation; Halley C. Oliveira for conceptualization, funding acquisition, project administration, resources, validation, and writing review and editing; José Antonio Pimenta for conceptualization, funding acquisition, project administration; Liliane Marcia M. Henning for conceptualization, funding acquisition, resources; Luiz Alberto Colnago for formal analysis, funding acquisition, resources; Renata Stolf-Moreira for conceptualization, funding acquisition, project administration, resources, supervision, validation, and writing review and editing.

References

1. Myers, N.; Mittermeier, R. A.; Mittermeier, C. G.; da Fonseca, G. A. B.; Kent, J.; *Nature* **2000**, *403*, 853.
2. Albino, B. É. S.; Canatto, R. A.; Cordeiro, A. T.; Fukushima, C. H.; Pilon, A. M.; *Rev. Bras. Eng. Biosistemas* **2019**, *13*, 88.
3. ITIS Species 2000; *Catalogue of Life: 2019 Annual Checklist*, available at <http://www.catalogueoflife.org/annual-checklist/2019/search/all/key/Cariniana/fossil/1/match/1/page/1/sort/direction/asc>, accessed in April 2022.
4. Ribeiro, R. V.; Bieski, I. G. C.; Balogun, S. O.; Martins, D. T. O.; *J. Ethnopharmacol.* **2017**, *205*, 69.

5. Hobbie, S. E.; Ogdahl, M.; Chorover, J.; Chadwik, O. A.; Oleksyn, J.; Zytowski, R.; Reich, P. B.; *Ecosystems* **2007**, *10*, 999.
6. Reis, J. R. M.; Fontoura, T.; *Biota Neotrop.* **2009**, *9*, 73.
7. Shimamoto, C. Y.; Botosso, P. C.; Marques, M. C. M.; *For. Ecol. Manage.* **2014**, *329*, 1.
8. Soares, J. A. H.; Souza, A. L. T.; Pestana, L. F. A.; Tanaka, M. O.; *Ecol. Eng.* **2020**, *152*, 105899.
9. Souza, P. P.; Resende, A. M. M.; Augusti, D. V.; Badotti, F.; Gomes, F. D. C. O.; Catharino, R. R.; Eberlin, M. N.; Augusti, R.; *Food Chem.* **2014**, *143*, 77.
10. Silva, R. M.; Ribeiro, R. T. M.; Souza, R. J. C.; Oliveira, A. F. M.; Silva, S. I.; Gallão, M. I.; *Acta Bot. Bras.* **2017**, *31*, 137.
11. Jovel, E. M.; Cabanillas, J.; Towers, G. H. N.; *J. Ethnopharmacol.* **1996**, *53*, 149.
12. Roumy, V.; Ruiz Macedo, J. C.; Bonneau, N.; Samaille, J.; Azaroual, N.; Encinas, L. A.; Rivière, C.; Hennebelle, T.; Sahpaz, S.; Antherieu, S.; Pinçon, C.; Neut, C.; Siah, A.; Gutierrez-Choquevilca, A. L.; Ruiz, L.; *J. Ethnopharmacol.* **2020**, *249*, 11241.
13. Bieski, I. G. C.; Leonti, M.; Arnason, J. T.; Ferrier, J.; Rapinski, M.; Violante, I. M. P.; Balogun, S. O.; Pereira, J. F. C. A.; Figueiredo, R. D. C. F.; Lopes, C. R. A. S.; Silva, D. R.; Pacini, A.; Albuquerque, U. P.; Oliveira-Martins, D. T.; *J. Ethnopharmacol.* **2015**, *173*, 383.
14. Baurin, N.; Arnoult, E.; Scior, T.; Do, Q. T.; Bernard, P.; Greenpharma, S. A.; *J. Ethnopharmacol.* **2002**, *82*, 155.
15. Lima Neto, G. A.; Kaffashi, S.; Luiz, W. T.; Ferreira, W. R.; Dias da Silva, Y. S. A.; Pazin, G. V.; Violante, I. M. P.; *Rev. Bras. Pl. Med.* **2015**, *17*, 1069.
16. Tiepo, A. N.; Constantino, L. V.; Madeira, T. B.; Gonçalves, L. S. A.; Pimenta, J. A.; Bianchini, E.; Oliveira, A. L. M.; Oliveira, H. C.; Stolf-Moreira, R.; *Planta* **2020**, *251*, 83.
17. Tiepo, A. N.; Hertel, M. F.; Rocha, S. S.; Calzavara, A. K.; Oliveira, A. L. M.; Pimenta, J. A.; Oliveira, H. C.; Bianchini, E.; Stolf-Moreira, R.; *Plant Physiol. Biochem.* **2018**, *130*, 277.
18. Oliveira-Filho, A. T.; Galetti, M.; *Primates* **1996**, *37*, 87.
19. Leite, E. J.; *Res. J. Bot.* **2007**, *2*, 138.
20. Guidugli, M. C.; Campos, T.; Sousa, A. C. B.; Feres, J. M.; Sebbenn, A. M.; Mestriner, M. A.; Contel, E. P. B.; Alzate-Marin, A. L.; *Conserv. Genet.* **2009**, *10*, 1001.
21. Silva, L. F.; Silva, M. L.; Cordeiro, S. A.; *Rev. Agroamb.* **2012**, *4*, DOI: 10.18406/2316-1817v4n22012448.
22. Janovik, V.; Boligon, A.; Athayde, M.; *Res. J. Phytochem.* **2012**, *6*, 105.
23. Janovik, V.; Boligon, A. A.; Frohlich, J. K.; Schwanz, T. G.; Pozzebon, T. V.; Alves, S. H.; Athayde, M. L.; *Nat. Prod. Res.* **2012**, *26*, 66.
24. Mabry, T. J.; Markham, K. R.; Thomas, M. B. In *The Systematic Identification of Flavonoids*, vol. 1, 1st ed.; Springer-Verlag: New York, USA, 1970.
25. García-Villalba, R.; Espín, J. C.; Tomás-Barberán, F. A.; Rocha-Guzmán, N. E.; *J. Food Compos. Anal.* **2017**, *63*, 38.
26. Schwarz, M.; Weber, F.; Durán-Guerrero, E.; Castro, R.; Rodríguez-Dodero, M. C.; García-Moreno, M. V.; Winterhalter, P.; Guillén-Sánchez, D.; *Foods* **2021**, *10*, 131.
27. Górecki, T.; Lynen, F.; Szucs, R.; Sandra, P.; *Anal. Chem.* **2006**, *78*, 3186.
28. Shukla, V.; Singh, P.; Kumar, D.; Konwar, R.; Singh, B.; Kumar, B.; *Phytomed. Plus* **2021**, *1*, 100025.
29. Ruan, J.; Yan, J.; Zheng, D.; Sun, F.; Wang, J.; Han, L.; Zhang, Y.; Wang, T.; *Molecules* **2019**, *24*, 2784.
30. *Chenomx NMR Suite*, version 9.0; Chenomx Inc., Edmonton, Alberta, Canada, 2020.
31. Hussin, N. M.; Muse, R.; Ahmad, S.; Ramli, J.; Mahmood, M.; Sulaiman, M. R.; Shukor, M. Y. A.; Rahman, M. F. A.; Aziz, K. N. K.; *Afr. J. Biotechnol.* **2009**, *8*, 2835.
32. Ferreira, E. L. F.; Mascarenhas, T. S.; Oliveira, J. P. C.; Chaves, M. H.; Araújo, B. Q.; Cavalheiro, A. J.; *J. Med. Plants Res.* **2014**, *8*, 353.
33. Janovik, V.; Boligon, A. A.; Frohlich, J. K.; Schwanz, T. G.; Pozzebon, T. V.; Alves, S. H.; Athayde, M. L.; *Nat. Prod. Res.* **2010**, *26*, 67.
34. Adiele, L. C.; Adiele, R. C.; Enye, J. C.; *Asian Pac. J. Trop. Med.* **2014**, *1*, 620.
35. Obafemi, T. O.; Onasanya, A. L.; Afolabi, B. A.; Falode, J. A.; Adeoye, A. O.; Afolabi, O. B.; Awe, J. O.; Fadaka, A. O.; Ochuko, O.; Jacdonmi, T.; Boligon, A. A.; Athayde, M. L.; *Pharmacology online* **2016**, *110*, 109.
36. Nguyen, V.; Nguyen, T. L.; Tran, H. T.; Ha, T. A.; Bui, V. H.; Nguyen, H. N.; Nguyen, T. D.; *Syst. Ecol.* **2014**, *55*, 219.
37. Iwashina, T.; Kokubugata, G.; *Bull. Natl. Mus. Nat. Sci., Ser. E* **2016**, *42*, 41.
38. Mierziak, J.; Kostyn, K.; Kulma, A.; *Molecules* **2014**, *19*, 16240.
39. Sytar, O.; Hemmerich, I.; Zivcak, M.; Rauh, C.; Brestic, M.; *Saudi J. Biol. Sci.* **2018**, *25*, 631.
40. Spiridon, I.; Colceru, S.; Anghel, N.; Teaca, C. A.; Bodirlau, R.; Armatu, A.; *Nat. Prod. Res.* **2011**, *25*, 1657.

Submitted: December 6, 2021

Published online: June 14, 2022

