

CHEMICAL CONSTITUENTS FROM THE LEAVES OF *Eriodictyon californicum* (Hook. & Arn.) Torr. (Boraginaceae)

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Eriodictyon californicum is a species belonging to Boraginaceae family, popularly known as “yerba santa” and used in folk medicine to treat rheumatism, headaches, inflammation, and respiratory complications. In the present work, we performed phytochemical analyses of extracts from the leaves of *E. californicum* in order to isolate its chemical constituents. According to thin-layer chromatography (TLC) analyses, preliminary phytochemical screening indicated the presence of anthocyanins derivatives, flavonoids, condensed and hydrolysable tannins. Furthermore, classical chromatographic techniques (column chromatography, analytical and preparative thin-layer chromatography) led to the isolation of secondary metabolites. The compounds were elucidated by 1D and 2D nuclear magnetic resonance (NMR) (correlation spectroscopy (COSY), heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC)) analysis, Fourier transform infrared (FTIR) spectroscopy, mass spectrometry (MS) analysis, and comparison with literature data. In this study, seven metabolites were identified: sterubin (1), chrysoeriol (2), apigenin (3), hydroxygenkwanin (4), 5,4'-dihydroxy-7,3'-dimethoxyflavanone (5), ethyl cinnamate (6) and 5-hydroxy-7-methoxychromone (7). The compounds 1-5 were previously identified in this species, and the compounds 6 and 7 are being reported for the first time in the species *E. californicum*, as well as in Boraginaceae family. The ¹H and ¹³C NMR data for some compounds have been reviewed. These results revealed that *E. californicum* is an important source of secondary metabolites, mainly flavonoids with chemophenetic relationships with Boraginaceae family.

Keywords: Boraginaceae; *Eriodictyon californicum*; flavonoids; phytochemistry.

INTRODUCTION

The Boraginaceae family comprises approximately 2000 species, which can be found in more than 100 genera.¹ Medicinally, the biological activities of species of this family are related to the presence of bioactive compounds, mainly flavonoids, and polyphenols.^{1,2} Among the genera of this family, the genus *Eriodictyon* comprises about 10 species restricted to the southwestern United States and northern Baja California, Mexico.^{3,4}

Eriodictyon californicum, a typical medicinal plant of the Boraginaceae family and employed in the treatment of skin wounds is popularly known as “yerba santa”. Its leaves are used by indigenous American tribes in the cure of several diseases, some directly associated with aging, including rheumatism, headaches, and inflammation, in addition to respiratory complications such as asthma, cough, and pulmonary infections.^{4,5} Nowadays, this species is available for use as a dietary supplement and homeopathic medicine for the supportive treatment of asthma and bronchial diseases.⁶ Chemically, *E. californicum* is recognized by the presence of flavonoids as main secondary metabolites. Previous phytochemical investigation using high-performance liquid chromatography (HPLC)

describes that “yerba santa” is especially rich in polyphenols and flavonoids.⁷

Flavonoids are naturally occurring phenolic compounds abundantly present in plants synthesized as secondary metabolites and possess various functions, including antimicrobial and antioxidant agents, visual attractors, photoreceptors, feeding repellent, and light screening.^{8,9} Furthermore, flavonoids have wide therapeutic potential, and are found in several natural sources, such as leaves, fruits, bulbs, barks, stems, and roots.^{10,11} Some of the flavonoids identified in the literature¹²⁻¹⁴ exhibit pharmacological activities, such as antioxidant, anticancer, antimicrobial, anti-inflammatory, antihypertensive and neuroprotective actions. A research showed that extracts and compounds isolated from *E. californicum* have pharmacological properties, such as anti-inflammatory,^{4,15,16} antioxidant,^{6,15} potential cancer chemopreventive,¹⁵ antibacterial¹⁷ and neuroprotective activities.^{3,4,16,18} Also, in the past, this species was used to mask the taste of quinine and other bitter medicines, thus, studies have evaluated taste modulating properties of extracts or isolated compounds in the species.^{7,19-21}

In this work, moving forward in the study of the chemical and pharmacological significance of *Eriodictyon californicum*, we report the isolation and identification of phenolic compounds from the leaves of *E. californicum*, metabolites with chemophenetic importance to the Boraginaceae family. The ¹H and ¹³C nuclear magnetic resonance (NMR) data for some compounds have been reviewed.

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EXPERIMENTAL

General experimental procedures

The Fourier-transform infrared (FTIR) spectra were acquired in KBr pellets on a Shimadzu spectrometer (IRTracer-100) in mid-IR, equipped with a Universal ATR (attenuated total reflectance) sampling device containing diamond/ZnSe crystal. Spectra were acquired and then processed in the OriginPro 8 (OriginLab Corporation, Massachusetts, United States). The spectra were scanned at room temperature in transmission mode over the wavenumber range of 4000-400 cm^{-1} , with 100 accumulations at a resolution of 4 cm^{-1} . A background spectrum was scanned under the same instrumental conditions before each series of measurements.

One-dimensional (1D) and two-dimensional (2D) NMR experiments were performed in DMSO- d_6 (dimethyl sulfoxide) or CDCl_3 at 298 K on a Bruker™ ASCEND III 400 NMR operating at 9.4 T, observing ^1H and ^{13}C at 400 and 100 MHz, respectively. One-bond ^1H - ^{13}C (heteronuclear single quantum correlation (HSQC)) and long-range (heteronuclear multiple bond correlation (HMBC)) NMR correlation experiments were optimized for an average coupling constant of $^1J_{\text{C,H}}$ and $^{1R}J_{\text{C,H}}$ of 140 and 8 Hz, respectively. All ^1H and ^{13}C NMR chemical shifts (δ) are expressed in ppm related to the tetramethylsilane (TMS) signal at 0.00 ppm as an internal reference, and the coupling constants (J) in Hz.

Mass spectrometric (MS) analysis used a TSQ Quantum Access spectrometer, equipped with an atmospheric pressure chemical ionization (APCI) source and operating in positive and negative acquisition mode. Samples (1 mg mL^{-1}) were prepared in methanol. MS/MS spectra were obtained from the application of energy from 25 to 35 eV. To obtain the mass spectra by direct injection, an Ion Trap-amaZonX spectrometer (Bruker) was used for electrospray ionization-mass spectrometry (ESI-MS, low resolution) and a micrOTOF II (Bruker) for high-resolution electrospray ionization-mass spectrometry (HRESIMS, high resolution) operating with a capillary voltage of 3.5 kV, ESI in positive mode, end plate offset of 500 V, nebulizer 8.0 psi, dry gas (N_2) with a flow rate of 5.0 L h^{-1} and a temperature of 200 $^\circ\text{C}$. The spectra (m/z 50-1000) were recorded

every 2 s. Melting point (m.p.) of the isolated compounds were measured on a melting point apparatus (QUIMIS, model Q340S23), with temperature ranging from 0-310 $^\circ\text{C}$.

Plant material

Leaves of *E. californicum* were collected at Embrapa Semiárido on Bebedouro Experimental Field, in Petrolina (coordinates: 09 $^\circ$ 23'35" S, 40 $^\circ$ 30'27" W), state of Pernambuco, Brazil, in February 2020. The identity of the plant was confirmed by Prof. Dr. Lúcia Helena Piedade Kiill, a botanist of the Embrapa Semiárido, based on comparison with a voucher specimen (#HTSA 7850) deposited in the Herbário do Trópico do Semiárido (HTSA). All procedures for access to genetic patrimony and associated traditional knowledge were carried out, and the project was registered in SisGen (Register #A7FE154).

Extraction

The dried and powdered leaves of *E. californicum* (611.6 g) were subjected to maceration at room temperature using 95% ethanol as solvent. Three extractions were performed, renewing the solvent every 72 h, until the drug was completely depleted. The extractive solutions were filtered and concentrated in a rotatory evaporator at reduced pressure (40-50 $^\circ\text{C}$), in order to afford 39.9 g of crude ethanolic extract.

Preliminary phytochemical analysis

The qualitative presence of different secondary metabolites of crude ethanolic extract was evaluated by thin-layer chromatography (TLC). An aliquot of the extract solutions was applied in plates of silica gel 60 F₂₅₄ in aluminum supports (Merck, Darmstadt, Germany), eluted in different solvent systems, and applied in specific revelators for each secondary metabolite class, following protocols (Table 1).²² The plates were visualized in a UV camera at 254 and 365 nm. The evaluation of the phytochemical presence in the extracts was based on the spot profiles and comparison with reference standards.

Table 1. Elution systems and revelators used to characterize the main secondary metabolites by TLC

Phytochemical	Elution system (v/v)	Standard	Revelator
Anthocyanins	ethyl acetate:formic acid:acetic acid:water (100:11:11:26)	methylene blue	sulfuric anisaldehyde
Anthracene derivatives	ethyl acetate:methanol:water (100:13.5:10)	aloin	10% ethanolic KOH
Anthraquinones	petroleum ether:ethyl acetate:formic acid (75:25:1)	anthraquinone	phosphomolybdic acid / 10% ethanolic H_2SO_4
Condensed tannins	ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:26)	catechin epicatechin	hydrochloric vanillin
Coumarins	toluene:ethyl ether (1:1, saturated with acetic acid 10%)	scopoletin	10% ethanolic KOH
Hydrolysable tannins	<i>n</i> -butanol:acetone:phosphate buffer (40:50:10)	gallic acid tannic acid	ammonium iron (II) sulfate (1%)
Flavonoids	ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:26)	rutin quercetin	NEU reagent
Lignans	chloroform:methanol:water (70:30:4)	linseed extract	sulfuric vanillin
Naphthoquinones	toluene:formic acid (99:1)	lapachol	10% ethanolic KOH
Saponins	chloroform:glacial acetic acid:methanol:water (64:32:12:8)	saponin	sulfuric anisaldehyde
Triterpenes and steroids	toluene:chloroform:ethanol (40:40:10)	lupeol sitosterol	Liebermann-Burchard
Xanthines	ethyl acetate:methanol:water (100:13.5:10)	caffeine	iodine-KI-HCl

KOH: potassium hydroxide; H_2SO_4 : sulfuric acid; KI: potassium iodide; HCl: hydrochloric acid; NEU: Neu reagent; TLC: thin-layer chromatography.

Isolation of compounds

The crude ethanolic extract from the leaves of *E. californicum* (38.2 g) was submitted to vacuum liquid chromatography (VLC) with hexane (Hex, 900 mL), chloroform (CHCl₃, 900 mL), ethyl acetate (EtOAc, 900 mL), and methanol (MeOH, 900 mL) as the mobile phases, and silica gel 60 (70-230 mesh) as the stationary phase, giving hexanic (0.1 g), chloroformic (4.9 g), ethyl acetate (18.5 g), and methanolic (3.5 g) fractions, respectively. First, a precipitate on EtOAc fraction was successively washed with methanol and filtered, yielding a white powder. Qualitative TLC analyses confirm purity, giving compound **1** (2.0 g). The EtOAc fraction (7.7 g) was subjected to column chromatography (CC) using silica gel 60 as the stationary phase and as eluents hexane, CHCl₃, EtOAc and MeOH, individually or in binary mixtures in ascending order of polarity, affording 108 fractions (100 mL each), that after collected were concentrated in a rotary evaporator. The fractions were evaluated and pooled according to TLC analysis, using the solvent system Hex:EtOAc (3:7), yielding 14 groups (G1 to G14). After the precipitation of G7, the precipitate was washed with methanol, giving a mixture of **1**, **2**, and **3** (40.4 mg). Similarly, the precipitate of group G8 was submitted to the same procedure as the before fraction, yielding a mixture of **2** and **4** (61.3 mg). The CHCl₃ fraction (4.9 g) was subjected to CC in the same conditions as described above, yielding 123 fractions, that were pooled in 26 groups (G1 to G26) according to TLC analysis. The precipitate of group G21 (12.1 mg) was successively washed with methanol, resulting in a white amorphous solid, affording **5** (12.1 mg), and qualitative TLC analysis confirmed purity. Groups G10 (2.8 mg) and G17 (7.8 mg), giving compounds **6** (2.8 mg) and **7** (7.8 mg), respectively.

Spectral data

Sterubin (**1**)

White amorphous powder; m.p.: 220-223 °C; ESI-MS: [M + H]⁺ *m/z*: 303.0863 (calcd. *m/z* 303.0863, error: 0.1 ppm), molecular formula C₁₆H₁₄O₆; FTIR (KBr) ν_{\max} / cm⁻¹ 3190 (OH), 2943, 2850 (C-H, aliphatic), 1639 (C=O, enolic), 1303, 1118 (C-O), 1604, 1454, 860, 732 (C=C, aromatic); ¹H NMR (400 MHz, DMSO-*d*₆) δ 6.89 (*br s*, 1H, H-2'), 6.76 (*br s*, 2H, H-5' and H-6'), 6.09 (*d*, 1H, *J* 2.4 Hz, H-8), 6.07 (*d*, 1H, *J* 2.2 Hz, H-6), 5.42 (*dd*, 1H, *J* 12.5 and 3.0 Hz, H-2), 3.78 (*s*, 3H, OCH₃-7), 3.24 (*dd*, 1H, *J* 17.2 and 12.5 Hz, H-3 α), 2.72 (*dd*, 1H, *J* 17.2 and 3.0 Hz, H-3 β); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 197.39 (C-4), 167.87 (C-7), 163.66 (C-5), 163.29 (C-9), 146.24 (C-4'), 145.68 (C-3'), 129.74 (C-1'), 118.45 (C-6'), 115.82 (C-5'), 114.85 (C-2'), 103.08 (C-10), 95.04 (C-6), 94.24 (C-8), 79.12 (C-2), 56.33 (OCH₃-7), 42.59 (C-3).

Chrysoeriol (**2**)

ESI-MS: [M + H]⁺ *m/z*: 301.0704 (calcd. *m/z* 301.0707, error: 1.0 ppm), molecular formula C₁₆H₁₂O₆; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.99 (*s*, 1H, OH-5), 7.55 (*m*, 2H, H-2' and H-6'), 6.96 (*m*, 1H, H-5'), 6.88 (*s*, 1H, H-3), 6.51 (*d*, 1H, *J* 2.0 Hz, H-8), 6.22 (*d*, 1H, *J* 2.0 Hz, H-6), 3.92 (*s*, 3H, OCH₃-3'); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 182.24 (C-4), 164.56 (C-7), 164.08 (C-2), 161.91 (C-5), 157.78 (C-9), 151.15 (C-4'), 148.45 (C-3'), 122.02 (C-1'), 120.77 (C-6'), 116.21 (C-5'), 110.56 (C-2'), 104.20 (C-10), 103.65 (C-3), 99.28 (C-6), 94.48 (C-8), 56.36 (OCH₃-3').

Apigenin (**3**)

ESI-MS: [M + H]⁺ *m/z*: 271.0602 (calcd. *m/z* 271.0601, error: -0.3 ppm), molecular formula C₁₅H₁₀O₅; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.98 (*s*, 1H, OH-5), 7.91 (*d*, 2H, *J* 8.7 Hz, H-2' and

H-6'), 6.95 (*d*, 2H, *J* 8.8 Hz, H-3' and H-5'), 6.75 (*s*, 1H, H-3), 6.48 (*d*, 1H, *J* 2.2 Hz, H-8), 6.22 (*d*, 1H, *J* 2.0 Hz, H-6); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 182.18 (C-4), 164.16 (C-7), 163.96 (C-2), 161.93 (C-5), 161.60 (C-4'), 157.76 (C-9), 128.87 (C-2' and C-6'), 121.67 (C-1'), 116.41 (C-3' and C-5'), 103.27 (C-3), 103.94 (C-10), 99.33 (C-6), 94.41 (C-8).

Hydroxygenkwanin (**4**)

ESI-MS: [M + H]⁺ *m/z*: 301.0702 (calcd. *m/z* 301.0707, error: 1.6 ppm), molecular formula C₁₆H₁₂O₆; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.98 (*s*, 1H, OH-5), 7.45 (*m*, 2H, H-2' and H-6'), 6.93 (*m*, 1H, H-5'), 6.70 (*s*, 1H, H-3), 6.67 (*d*, 1H, *J* 2.0 Hz, H-8), 6.35 (*d*, 1H, *J* 2.0 Hz, H-6), 3.87 (*s*, 3H, OCH₃-3'); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 182.22 (C-4), 165.49 (C-7), 164.64 (C-2), 161.66 (C-5), 157.60 (C-9), 150.25 (C-4'), 146.21 (C-3'), 121.90 (C-1'), 119.50 (C-6'), 116.41 (C-5'), 113.95 (C-2'), 105.09 (C-10), 103.48 (C-3), 98.31 (C-6), 92.91 (C-8), 56.38 (OCH₃-3').

5,4'-Dihydroxy-7,3'-dimethoxyflavanone (**5**)

White amorphous solid; FTIR (KBr) ν_{\max} / cm⁻¹ 3414 (OH), 2981, 2850 (C-H, aliphatic), 1639 (C=O, enolic), 1296, 1157 (C-O), 1573, 1400, 840, 744 (C=C, aromatic); ¹H NMR (400 MHz, CDCl₃) δ 12.02 (*s*, 1H, OH-5), 6.97 (*m*, 1H, H-2'), 6.95 (*m*, 2H, H-5' and H-6'), 6.08 (*d*, 1H, *J* 2.3 Hz, H-6), 6.05 (*d*, 1H, *J* 2.3 Hz, H-8), 5.34 (*dd*, 1H, *J* 13.1 and 3.0 Hz, H-2), 3.93 (*s*, 3H, OCH₃-3'), 3.81 (*s*, 3H, OCH₃-7), 3.10 (*dd*, 1H, *J* 17.2 and 13.1 Hz, H-3 α), 2.79 (*dd*, 1H, *J* 17.2 and 3.0 Hz, H-3 β); ¹³C NMR (100 MHz, CDCl₃) δ 195.99 (C-4), 167.98 (C-7), 164.17 (C-5), 162.84 (C-9), 146.78 (C-3'), 146.24 (C-4'), 130.22 (C-1'), 119.62 (C-6'), 114.54 (C-5'), 108.77 (C-2'), 103.13 (C-10), 95.13 (C-6), 94.28 (C-8), 79.36 (C-2), 56.02 (OCH₃-3'), 55.70 (OCH₃-7), 43.41 (C-3).

Ethyl cinnamate (**6**)

¹H NMR (400 MHz, CDCl₃) δ 7.69 (*d*, 1H, *J* 16.1 Hz, H-7), 7.52 (*m*, 2H, H-2 and H-6), 7.38 (*m*, 2H, H-3 and H-5), 7.38 (*m*, 1H, H-4), 6.44 (*d*, 1H, *J* 16.1 Hz, H-8), 4.27 (*q*, 2H, *J* 7.2 Hz, H-10), 1.34 (*t*, 3H, *J* 7.2 Hz, H-11); ¹³C NMR (100 MHz, CDCl₃) δ 166.8 (C-9), 144.60 (C-7), 134.30 (C-1), 130.22 (C-4), 128.88 (C-3 and C-5), 128.1 (C-2 and C-3), 118.30 (C-8), 60.52 (C-10), 14.13 (C-11).

5-Hydroxy-7-methoxychromone (**7**)

¹H NMR (400 MHz, CDCl₃) δ 12.56 (*s*, 1H, OH-5), 7.74 (*d*, 1H, *J* 6.0 Hz, H-2), 6.39 (*d*, 1H, *J* 2.3 Hz, H-8), 6.37 (*d*, 1H, *J* 2.3 Hz, H-6), 6.22 (*d*, 1H, *J* 6.0 Hz, H-3), 3.86 (*s*, 3H, OCH₃-7); ¹³C NMR (100 MHz, CDCl₃) δ 183.30 (C-4), 165.20 (C-7), 162.70 (C-5), 158.20 (C-9), 155.62 (C-2), 111.41 (C-3), 106.70 (C-10), 98.25 (C-6), 92.75 (C-8), 56.06 (OCH₃-7).

RESULTS AND DISCUSSION

The phytochemical analysis of plants presents an idea about the chemical nature of compounds that confer host plants with certain effective biological activity.²³ This analysis is a qualitative method carried out with the aim of identifying the possible chemical constituents present in the vegetal materials from revealers that indicate certain classes of secondary metabolites. Thus, secondary metabolites not only play a vital role in plant defense against environmental stresses but also serve as therapeutic components for humans.²⁴ The results of the phytochemical analysis from leaves of *E. californicum* are shown in Table 2.

The analysis indicated positive reaction to the presence of anthocyanins, flavonoids, condensed and hydrolysable tannins. Some factors regulate the production of leaf phytochemicals, such

Table 2. Phytochemical characterization of the extract of *Eriodictyon californicum* leaves

Phytochemical	Crude ethanolic extract of <i>E. californicum</i>
Anthocyanins	+++
Anthracene derivatives	++
Anthraquinones	++
Condensed tannins	+++
Coumarins	++
Hydrolysable tannins	+++
Flavonoids	+++
Lignans	+
Naphthoquinones	-
Saponins	++
Triterpenes and steroids	-
Xanthines	++

(-): not detected; (+): weak presence; (++): moderate presence; (+++): strong presence.

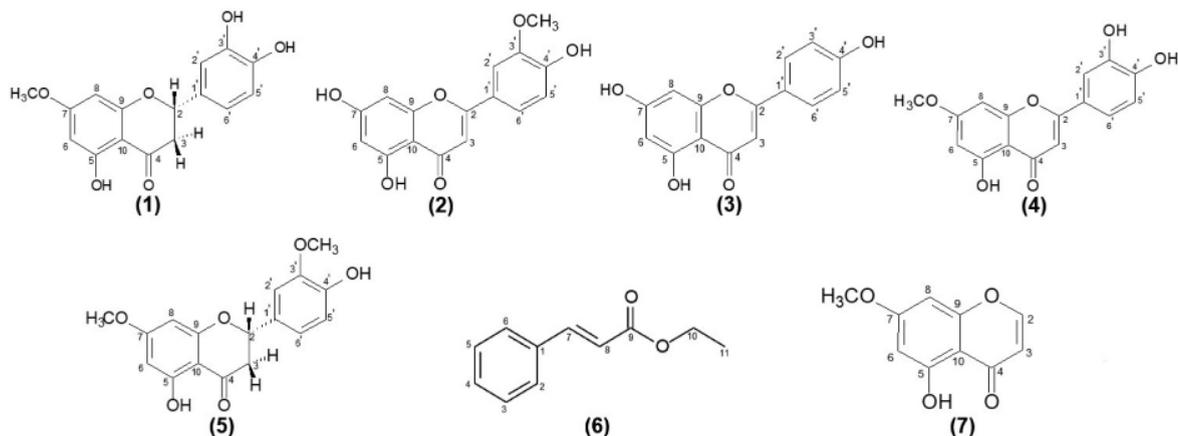
as soil nutrients, water or light availability, as well can influence plant phytochemistry by modulating the costs associated with their production and deployment of phytochemicals.²⁵

The phytochemical investigation of the ethyl acetate and chloroform fractions from the leaves of *E. californicum* affording seven metabolites, including five flavonoids named sterubin (**1**),²⁶ chrysoeriol (**2**),²⁷ apigenin (**3**),²⁸ hydroxygenkwanin (**4**)²⁹ and 5,4'-dihydroxy-7,3'-dimethoxyflavanone (**5**),³⁰ in addition to other two uncommon metabolites, one phenylpropanoid known as ethyl cinnamate (**6**)³¹ and one chromone identified as 5-hydroxy-7-methoxychromone (**7**).³² Compounds **1-5** were previously found in this species, whereas compounds **6** and **7** are reported for the first time in *E. californicum* and in the Boraginaceae family. The complete ¹H and ¹³C NMR data for these compounds were reviewed according to 1D and 2D NMR experiments (COSY, HSQC and HMBC), when possible, in combination with FTIR and ESI-MS, as well as by comparison with literature data.²⁶⁻³² The chemical structures of the compounds are presented in Figure 1. Although the structures of the compounds **6** and **7** have already been described a long time ago, their ¹H and ¹³C NMR data are incomplete or scalar coupling constants values have not been assigned. In this work, the complete and unequivocal ¹H and ¹³C NMR data were reviewed according to 1D and 2D NMR experiments (see Tables 1S-7S in the Supplementary Material).

Compound **1** was obtained as a white amorphous powder with the molecular formula C₁₆H₁₄O₆ determined by ESI-MS (observed *m/z* 303.0863 [M + H]⁺) and NMR analyses. Infrared (IR) spectrum showed absorption bands at 3190, 2943-2850 and 1639 cm⁻¹ typical of hydroxyl, C-H sp³, and enolic carbonyl groups. In addition, absorption bands at 1303-1118 cm⁻¹ were related to C-O of phenols or aromatic ethers, while deformations bands at 1604-1454 and 860-732 cm⁻¹ were indicative of the presence of an aromatic ring. The atmospheric-pressure chemical ionization (APCI)-MS spectrum of **1** displayed fragments in positive and negative modes, highlighting the retro-Diels-Alder (RDA) reaction on C-ring of the structure, followed by rearrangement. The main fragment pathway of flavonoids within aglycone is the RDA reaction coupled to losses of small neutral molecules and fragments.³³ MS/MS displayed fragmentation pattern similar to the literature.⁴ The ¹H NMR spectrum exhibited characteristic signals of methylene and methine hydrogens at δ_H 2.72 (*dd*, 1H, *J* 17.2 and 3.0 Hz, H-3β), δ_H 3.24 (*dd*, 1H, *J* 17.2 and 12.5 Hz, H-3α) and δ_H 5.42 (*dd*, 1H, *J* 12.5 and 3.0 Hz, H-2), characteristic of flavanones. Furthermore, it was observed two doublets at δ_H 6.07 (*d*, 1H, *J* 2.2 Hz, H-6) and δ_H 6.09 (*d*, 1H, *J* 2.2 Hz, H-8), coupled in *meta*, representative of aromatic hydrogens of the flavanone A-ring and suggesting the existence of a 6,8-disubstituted ring. Two broad singlets at δ_H 6.76 (*br s*, 2H, H-5' and H-6') and δ_H 6.89 (*br s*, 1H, H-2'), indicated the presence of a trisubstituted B ring flavonoid in the structure. The singlet at δ_H 3.78 (*s*, 3H, OCH₃-7) revealed the presence of a methoxyl group.

¹³C DEPTQ (distortionless enhancement by polarization transfer with quaternary carbon detection) NMR spectrum, as well as one-bond and long-range ¹H-¹³C correlations from HSQC and HMBC NMR experiments, indicated a total of 16 atoms of carbon, which comprised typical signals of aromatic carbons at δ_C 94.24, 95.04, 103.08, 114.85, 115.82, 118.45, 129.74, 145.68, 146.24, 163.29, 163.66 and 167.87, one methine carbon at δ_C 79.12 assigned to a saturated oxygenated, one methylene carbon at δ_C 42.59, one carbonyl carbon at δ_C 197.39, and finally one carbon at δ_C 56.33, confirming the presence of a methoxyl group.

The hydrogen at δ_H 5.42 (*dd*, 1H, *J* 12.5 and 3.0 Hz, H-2) showed direct ¹H-¹³C HSQC correlation map with the carbon at δ_C 79.12 and long-range ¹H-¹³C correlations with the carbons at δ_C 114.85 (C-2'), 118.45 (C-6'), 129.74 (C-1'), and 197.39, which supports the substitution pattern proposed with the carbonyl group located at C-4, characteristic of C-ring of the flavanone. On the other hand, the methylene hydrogens showed one bond ¹H-¹³C HSQC correlation map with the carbon at δ_C 42.6 and long-range ¹H-¹³C correlations with the carbons at δ_C 79.12 (C-2), 103.08 (C-10), 129.74 (C-1'), and 197.39, confirming the methylene group at C-3.

**Figure 1.** Chemical constituents isolated from the leaves of *Eriodictyon californicum*

The location of the methoxyl group at C-7 was established due to the singlet at δ_{H} 3.78 with long-range ^1H - ^{13}C correlation map from HMBC NMR experiment with the carbon at δ_{C} 167.87. Moreover, the doublets at δ_{H} 6.09 (H-8) and 6.07 (H-6) revealed long-range ^1H - ^{13}C correlation map from HMBC NMR experiment with the carbon at δ_{C} 167.9 (C-7). Also, the hydroxyl group at position C-5 was observed due to the long-range ^1H - ^{13}C correlation map from HMBC NMR experiment with the carbons at δ_{C} 95.04 (C-6), 103.08 (C-10), and 163.66 (C-5). In contrast, the presence of other hydroxyl groups in the molecule in the B ring at C-3' and C-4' were established on the basis of long-range ^1H - ^{13}C correlation map from HMBC NMR experiment of the hydrogens at δ_{H} 6.76 (H-5 and H-6') and 6.89 (H-2') with the carbon δ_{C} 145.68 (C-3') and 146.24 (C-4'), respectively, which are typical of the aromatic B-ring. Thus, according to the IR, MS and ^1H and ^{13}C NMR 1D/2D data, and comparison with literature data, compound **1** was identified as a flavanone sterubin, a chemophenetic marker of the genus *Eriodictyon*. This compound possesses neuroprotective activity, mainly against Alzheimer disease.^{3,18}

^1H and ^{13}C NMR data analyses of G7 in comparison with literature data, resulted in the identification of a mixture of metabolites named sterubin (**1**),²⁶ chrysoeriol (**2**)²⁷ and apigenin (**3**).²⁸ On the other hand, ^1H and ^{13}C NMR data of G8, resulted the mixture of flavones known as chrysoeriol (**2**) and hydroxygenkwanin (**4**).²⁹ The G21 resulted in the identification of 5,4'-dihydroxy-7,3'-dimethoxyflavanone (**5**).³⁰ All these flavonoids have been previously reported in *E. californicum*. While sterubin (**1**) is a chemophenetic marker of the genus *Eriodictyon*,⁴ chrysoeriol (**2**) has been identified in the species *E. angustifolium*,^{20,34,35} *E. californicum*^{15,20,35-37} and *E. trichocalyx*,³⁸ apigenin (**3**) was isolated from *E. angustifolium*, *E. californicum*, *E. tomentosum*,³⁴ *E. trichocalyx*³⁸ and *E. sensifolium*,³⁹ the compound hydroxygenkwanin (**4**) was found in *E. angustifolium* and *E. californicum*,³⁵ and 5,4'-dihydroxy-7,3'-dimethoxyflavanone (**5**) was identified only in *E. californicum*.³⁷

The ^1H NMR spectrum of compound **6** revealed the presence of signals at δ_{H} 1.28 (*t*, 3H, *J* 7.2 Hz, CH_3) and 4.27 (*q*, 2H, *J* 7.2 Hz, OCH_2), assigned to an ethoxyl group (OCH_2CH_3). Additionally, the signals at δ_{H} 7.39 (*m*, 3H, H-3, H-5, and H-4) and 7.52 (*m*, 2H, H-2 and H-6) were attributed to the chemically equivalent aromatic hydrogens, suggesting the presence of a monosubstituted aromatic ring. The signals at δ_{H} 6.44 (*d*, 1H, *J* 16.1 Hz, H-8) and 7.69 (*d*, *J* 16.1 Hz, 1H, H-8) indicate a relative *trans* configuration between vicinal olefinic protons. The ^{13}C NMR spectrum showed 7 signals referring to 9 carbons. The signals at δ_{C} 14.14 and 60.51 were attributed to terminal methyl of the ethoxy group and oxymethylene carbon, respectively. Also, it was observed the presence of the signals at δ_{C} 118.30 (C-8) and 144.61 (C-7) for the olefinic carbons, and characteristic signals at δ_{C} 128.07 (C-2 and C-6), 128.89 (C-3 and C-5) and 130.23 (C-4) in the aromatic region. Non-hydrogenated carbons were determined through the long-range ^1H - ^{13}C correlation map from HMBC NMR experiment.

The ^1H - ^1H COSY correlation map of **6** showed coupling of signals at δ_{H} 1.28 (H-11)/ δ_{H} 4.27 (H-10), correlations between aromatic hydrogens δ_{H} 7.39 (H-3 and H-5; H-4)/ δ_{H} 7.52 (H-2 and H-6), and also of vicinal olefinic protons at δ_{H} 6.44 (H-8)/ δ_{H} 7.69 (H-7). The direct ^1H - ^{13}C correlation map from HSQC NMR experiment map allowed establishing the one-bond correlation between the signals at δ_{H} 7.52 (*m*, 2H,)/ δ_{C} 128.1 (C-2 and C-6), δ_{H} 7.39 (*m*, 2H)/ δ_{C} 128.9 (C-3 and C-5) and δ_{H} 7.39 (*m*, 1H)/ δ_{C} 130.2 (C-4), associated to the aromatic system. The long-range ^1H - ^{13}C HMBC correlation map showed the correlations of the olefinic hydrogens at δ_{H} 7.69 (H-7)/ δ_{C} 166.80 (C-9, J^2), characteristic to an ester carbonyl, and at δ_{H} 6.44 (H-8) with the non-hydrogenated aromatic carbon at δ_{C} 134.30 (C-1, J^3) and 166.80 (C-9, J^2). The correlation map also showed the

signal at δ_{H} 4.26 (H-10)/ δ_{C} 14.14 (C-11, J^2) and δ_{C} 166.80 (C-9, J^2), confirming that the ethoxy group is attached to the carbonyl carbon. These and another key correlation were illustrated in Figure 2. Comparison with the literature data³¹ allowed to identify compound **6** as the phenylpropanoid ethyl (*E*)-3-phenylprop-2-enoate, popularly known as ethyl cinnamate.

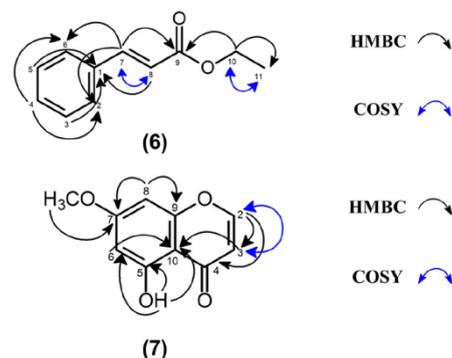


Figure 2. Key HMBC and COSY correlations of the compounds **6** and **7**

The ^1H NMR spectrum of compound **7** showed the signals at δ_{H} 7.74 (*d*, 1H, *J* 6.0 Hz, H-2) and 6.22 (*d*, 1H, *J* 6.0 Hz, H-3) for unsaturated methine hydrogens with vicinal coupling, and two doublets in δ_{H} 6.37 (*d*, 1H, *J* 2.3 Hz, H-6) and 6.39 (*d*, 1H, *J* 2.3 Hz, H-8), typical of the aromatic hydrogens on ring A. The signal at δ_{H} 3.86 (*s*, 3H, OCH_3 -7), suggested the presence of the methoxyl group and the signal at δ_{H} 12.56 (*s*, 1H, OH-5), was assigned to a hydroxyl group. The ^{13}C NMR spectrum (100 MHz, CDCl_3) revealed the presence of 5 carbons signals, among which, the signal in δ_{C} 56.34 indicated a free methoxyl. The signals at δ_{C} 92.77 (C-8) and 98.26 (C-6) are characteristic of the aromatic carbons and the signals at δ_{C} 111.43 (C-3) and 155.65 (C-2) were assigned to unsaturated carbons of the *cis* configuration of the molecule. Further signals were determined based on two-dimensional analysis.

Through ^1H - ^1H COSY correlation map was observed couplings between the vicinal olefinic hydrogens at δ_{H} 6.22 (H-3)/ δ_{H} 7.74 (H-2), confirming the structure of the chromone skeleton. The long-range ^1H - ^{13}C HMBC correlation map allowed to observe long distance between δ_{H} 3.81 (OCH_3 -7)/ δ_{C} 165.2 (C-7, J^3), confirming the location of the methoxyl on ring A. The signal at δ_{H} 6.39 (*d*, 1H, *J* 2.3 Hz, H-8) correlated with the non-hydrogenated carbon at δ_{C} 106.7 (C-10, J^2), and with the carbons at δ_{C} 165.2 (C-7, J^3) and 158.2 (C-9, J^3), characteristic of oxygenated aromatic carbons. Besides that, revealed the correlations of the signal at δ_{H} 7.74 (H-2)/ δ_{C} 183.3 (C-4, J^3), coupling with a carboxylic carbon. These and another key correlation were illustrated in Figure 2. The comparison of the NMR data corroborate the identification of these constituent as the chromone 5-hydroxy-7-methoxychromone (**7**).³² This compound has been identified in another vegetal species, but is being reported for the first time in *Eriodictyon californicum* and in the Boraginaceae family.

CONCLUSIONS

The phytochemical study of *E. californicum* leaves resulted in the identification of seven compounds, including five flavonoids. These compounds were previously identified in this species. Besides these, it was identified cinnamate derivative and a chromone, reported for the first time in *E. californicum* and in Boraginaceae family. Thus, the work contributed to the expansion of the phytochemical knowledge of the genus *Eriodictyon* and of the Boraginaceae family through the isolation of a new compound from the species *E. californicum*.

SUPPLEMENTARY MATERIAL

Supplementary data associated with this article (Figures 1S-84S and Tables 1S-7S) can be found in the online version at <http://quimicanova.sbq.org.br> as PDF file, with free access.

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