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# Chemical Profile, Antioxidant and Antimicrobial Activities of *Combretum lanceolatum* Pohl ex Eichler. (Combretaceae)

***Evaldo S. Monção Filho,[a] Ruth Raquel S. Farias,[b] João Sammy N. Souza,[a] Carmem D. L. Campos,[c] Cristina A. Monteiro,[c] Éverton Leandro F. Ferreira,[d,e] Mônica T. Pupo,[d] Mariana Helena Chaves*[,a] and Gerardo M. Vieira Júnior*[,a]***

[a]*Laboratório de Produtos Naturais, Departamento de Química, Universidade Federal do Piauí, 64049-550 Teresina-PI, Brazil*

[b]*Departamento de Biologia, Universidade Estadual do Piauí, 64280-000 Campo Maior-PI, Brazil*

[c]*Departamento de Biologia, Instituto Federal de Educação, Ciência e Tecnologia do Maranhão, 65030-005 São Luís-MA, Brazil*

[d]*Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, 14040-903 Ribeirão Preto-SP, Brazil*

[e]*Universidade Federal do Vale do São Francisco, 64770-000 São Raimundo Nonato-PI, Brazil*

Compared to other species, components of *Combretum lanceolatum* are still with few data reported in the literature. This work describes the compounds identification, antioxidant and antimicrobial activities of *C. lanceolatum* twigs. Ethanolic extract (EECLT) from *C. lanceolatum* twigs was submitted to partition obtaining: hexane (HF), diethyl ether (EEF), ethyl acetate (EAF) and hydromethanolic (HMF) fractions. They were analyzed by gas chromatography-mass spectrometry, high performance liquid chromatography coupled to mass spectrometry and direct insertion mass spectrometry and evaluated for antioxidant assay (2,2-diphenyl-1-picrylhydrazyl (DPPH) method) and antimicrobial assay. Fifty-seven compounds were identified in the fractions. In antioxidant assay, EECLT and EAF demonstrated good antioxidant potential (half maximal effective concentration ($EC_{50}$): 57.9 and 45.4 μg $mL^{-1}$, respectively), better than the positive control butylated hydroxytoluene (BHT) ($EC_{50}$: 69.34 μg $mL^{-1}$). For antimicrobial assay, all fractions presented promising minimal inhibitory concentration (MIC). EECLT presented the best values against *Staphylococcus aureus* (125 μg $mL^{-1}$) and *Escherichia coli* (62.5 μg $mL^{-1}$) and better than the positive control fluconazole (8 and 16 μg $mL^{-1}$) against *Candida albicans* (3.9 μg $mL^{-1}$) and *Candida krusei* (15.6 μg $mL^{-1}$) strains. This study presents relevant chemical and biological information about *C. lanceloatum* expanding the knowledge of Brazilian flora.

**Keywords:** Combretaceae, *Combretum lanceolatum*, phenolic compounds, antioxidant activity, antimicrobial activity

## Introduction

Combretaceae family is part of the Myrtales order, comprising 20 genera and about 600 plant species, distributed in tropical and subtropical regions of the world, existing in America, Asia and Africa, with the last continent being its largest center of diversity.[1] The two most common genera of this family are *Combretum* and *Terminalia*, with about 370 and 200 species, respectively.[2] In Brazil, the family is represented by five genera and 64 species, with 13 species endemic.[3]

Several classes of secondary metabolites are described in *Combretum* genus, such as triterpenes, saponins, phenanthrenes, bibenzyls, stilbenes, flavonoids, tannins, and other aromatic compounds.[4,5] This genus is found in several phytogeographic domains, including Atlantic Forest, Pantanal, Amazon, Caatinga and Cerrado.[6]

The variety of secondary metabolites makes *Combretum* genus a rich source of bioactive compounds and

*e-mail: magela@ufpi.edu.br; mariana@ufpi.edu.br
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possesses many biological properties such as antioxidant, nephroprotective, anti-inflammatory, antimalarial, antidiabetic, antimicrobial, analgesic, and cytotoxic/antiproliferative activities.[7-19]

*Combretum lanceolatum* Pohl ex Eichler, commonly known as "*pombeiro-vermelho*" or "*remela de macaco*", occurs in Amazon, Pantanal, Caatinga, Cerrado and Atlantic Forest, with a good distribution from north to south of Brazil, found yet in Paraguay, mostly in wetland areas.[20,21] The steam-bark of *C. lanceolatum* has been traditionally used in herbal medicine as an infusion to aid in digestion.[22]

A great number of plants can contain antioxidant compounds, which have the capacity to neutralize free radicals, reducing the risk of chronic diseases.[23] Various methods can be used to estimate this activity potential of different types of compounds, such as free radical neutralization assays, hydrogen atom transfer, and metal chelation. Among these methods, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is widely used due to its effectiveness, and to be quicker and lower-cost method.[7]

Compared to other *Combretum* species, however, the components of *C. lanceolatum* are still with few data reported and only a small number of compounds have been identified.[24] Therefore, this research focuses in chemical characterization and evaluates the antioxidant and antimicrobial activities of the ethanolic extract and the hexane, diethyl ether, ethyl acetate and hydromethanolic fractions from *C. lanceolatum* twigs.

## Experimental

### Chemical and reagents

Analytical grade solvents (diethyl ether, ethanol, ethyl acetate, hexane and methanol) were purchased from Labsynth® (Diadema, São Paulo, Brazil). The ultrapure water was prepared by ultrapure water purification Master System MS2000 from Gehaka® (São Paulo, Brazil), and the high-performance liquid chromatography (HPLC) grade solvent methanol was purchased from Tedia Company Inc.® (Ohio, USA). The extract and fractions of liquid-liquid extraction were concentrated on Heidolph rotary evaporator Laborota 4000® (Darmstadt, Germany).

For antioxidant and antimicrobial assays, the 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), RPMI-1640 medium, morphilinepropanesulfonic acid (MOPS), ciprofloxacin (CPR) and fluconazole (FLZ) were purchased from Sigma-Aldrich® (Poole, UK). The Mueller-Hinton agar (MH), Sabouraud dextrose agar (SDA) and dimethyl sulfoxide (DMSO) were purchased in Merk® (Darmstadt, Germany) and resazurin in Thermo Fisher Scientific® (Waltham, USA).

### Extraction and obtaining of the fractions

The *C. lanceolatum* twigs were collected and identified by the biologist Ruth Raquel Soares de Farias (State University of Piauí) in Campo Maior City (Piauí State, Brazil) in July 2021. A voucher specimen with register number TEPB-32808 was deposited at the herbarium Graziella Barroso of the Federal University of Piauí (Campus Ministro Petrônio Portella, Teresina, Piauí, Brazil) and registered with AAB530D number in National System of Genetic Heritage and Associated Traditional Knowledge (SisGen). Approximately 500 g of *C. lanceolatum* twigs were dried (15 days at ambient temperature) and then powdered. The powdered material was successively extracted with ethanol at room temperature (six times, 72 h). After the removal of the residual solvent, it was obtained 16.3 g of ethanolic extract of *C. lanceolatum* twigs (EECLT) (3.27%).

A liquid-liquid extraction was performed with 13 g of EECLT. The extract was suspended in methanol / water (150 mL, 1:2, v/v) and the supernatant was partitioned successively with hexane (600 mL, twelve times with 50 mL), diethyl ether (500 mL, ten times with 50 mL) and ethyl acetate (200 mL, four times with 50 mL), yielding 1.6 g (12.3%) of hexane fraction (HF), 3.2 g (24.6%) of diethyl ether fraction (EEF), 1.9 g (14.6%) of ethyl acetate fraction (EAF), 4.2 g (32.3%) of hydromethanolic fraction (HMF) and also a precipitate 2.1 g (16.1%).

### GC-MS analysis

The hexane fraction was analyzed by gas chromatography-mass spectrometry (GC-MS) performed on a Shimadzu QP2020 with automatic injector AOC-20i Shimadzu with interface in a mass spectrometry. The utilized column was a ZB-5HT (30 m × 0.25 μm × 0.25 μm). The oven temperature was set at 140 °C for 5 min and raised to 320 °C at a rate of 3 °C $min^{-1}$, remaining at this temperature for 10 min. The injector temperature was 270 °C, using helium (99.999%) as the carrier gas at a flow rate of 1 mL $min^{-1}$, with pressure of 88.5 kPa, linear velocity of 37.8 cm $s^{-1}$, and the detector temperature was 300 °C. The mass spectrometry was set by an electron ionization (EI) at 70 eV, with interface at 280 °C and range of acquisition *m/z* 35-700 Da. In addition, the retention indices (RI) of a series of *n*-C8-C40 alkanes were used to distinguish the tentatively identified compounds. All mass

spectra were identified by comparison with NIST08 and WILEY 229 libraries.

### HPLC-ESI-QTOF-MS/MS analysis

The EEF, EAF and HMF fractions were analyzed by a high-performance liquid chromatography coupled to electrospray ionization and quadrupole time-of-flight mass spectrometry (HPLC-ESI-QTOF-MS/MS), using a Bruker Daltonics high performance liquid chromatography coupled to a mass spectrometer microTOF-Q II (Bruker Daltonics, Massachusetts, USA). The chromatographic analysis was performed on a C6-Phenyl analytical column Ascentis Express (4.6 × 100 mm, 5 µm) using a flow rate of 1.0 mL $min^{-1}$, an injection volume of 10 µL, and a gradient solvent system with aqueous (0.1% formic acid) methanol (5 to 100% methanol for 15 min and 100% methanol for 5 min). Samples were dissolved in methanol / water (7:3, v/v) at the concentration of 0.5 mg $mL^{-1}$. Trifluoroacetic acid (NA-TFA, 500 mg $L^{-1}$) was used to perform the equipment calibration. The high-voltage capillary was set at 4500 V, the nebulizer gas pressure of 2 bar, dry temperature source 200 °C, flow of drying gas of 9.0 L $min^{-1}$ in source surface, spectral rate of 3 Hz for $MS^1$ and 10 Hz to $MS^2$. For fragments acquisition of MS/MS, the most intense ions at $MS^1$ were selected for collision-induced dissociation. The acquisition was in negative ion mode and data analysis were realized in Bruker Compass Data analysis (version 4.3), provided by the manufacturer.

### DI-ESI-IT-$MS^n$ mass spectrometry analysis

All fractions obtained (with the exception of HF) were separately dissolved in methanol (HPLC grade) at a concentration of 0.1 mg $mL^{-1}$ and filtered through 0.22 µm pore size of polytetrafluoroethylene (PTFE) membrane. A volume of 2.0 mL was transferred to a vial and the insertion volume was measured and pumped by an appropriate syringe.

The EEF, EAF and HMF fractions were analyzed by direct insertion with electrospray ionization source and an ion trap analyzer mass spectrometry (DI-ESI-IT-$MS^n$) in a AmaZon X mass spectrometer (Bruker Daltonics, Bremen, Germany). For mass spectrometry parameters, the ESI ionization source was set in negative ion mode, with *m/z* range 100-1500, syringe flow 5.0 µL $min^{-1}$, high-voltage capillary 4.5 kV, flow of drying gas ($N_2$) 4.0 L $min^{-1}$, nebulizer pressure 14 psi and dry temperature source 230 °C. For $MS^n$ analysis, the parameters of signal amplitude, data acquisition time and radiofrequency were adjusted for each signal, in order to obtain a better spectral information from each precursor ion. The acquisition and data analysis were realized in the same software used in HPLC-ESI-QTOF-MS/MS analysis, described previously.

### Total phenolic content

The total phenolic content (TPC) of the EECLT was determined by spectrometry in the visible region using Folin-Ciocalteu method as described by Sousa *et al.*,[25] in triplicate. Aqueous sodium carbonate (15%) and methanolic solution of sample, at a concentration of 1.0 mg $L^{-1}$, were prepared and stored. A solution of 500 µL of Folin-Ciocalteu reagent was added to an aliquot of 100 µL of the sample solution, followed by 5.0 mL of distilled water and stirred for one minute. Then, 2.0 mL of sodium carbonate solution (15%) was added to the mixture and stirred for 30 s, the volume of 10 mL was completed with distilled water, with the reaction lead for two hours and the measurement of the absorbance was at a wavelength of 750 nm. For the blank, the same procedure was performed, replacing the sample solution for 100 µL of methanol. The determination of TPC levels was made by interpolating the absorbance of the samples against an analytical curve constructed with a standard of gallic acid (10 to 350 µg $mL^{-1}$) and expressed as $A = 0.1185c - 0.0453$, with a correlation coefficient linear of 0.999, which "*c*" is the concentration of gallic acid and "A" is the absorbance. TPC was expressed in milligrams of gallic acid equivalent *per* gram of sample (mg GAE $g^{-1}$).

### Antioxidant activity

DPPH assay of EECLT, EEF, EAF and HMF was determined according to a method already described in the literature.[25] A solution of DPPH was prepared at a concentration of 40 µg $mL^{-1}$, it was stocked and kept under refrigeration and protected from light. The solutions of the extract and fractions were prepared at concentrations of 250, 200, 150, 100, 50 and 25 µg $mL^{-1}$. The synthetic compound BHT was used as positive control. The antioxidant activity was determined by monitoring absorbance of the reaction mixture (0.3 mL of the sample solution and 2.7 mL of the stock solution of DPPH at the concentration of 40 µg $mL^{-1}$) at 516 nm in triplicate. The absorbance was carried out in 30 min. A mixture of methanol (2.7 mL) and methanolic extract solution at the concentrations tested (0.3 mL) was used as a blank.

The equation of the analytical curve used to determine the concentration of DPPH was: $A = 33.227c + 1.0607$, with "*c*" as the equivalent of DPPH concentration, "A" the absorbance obtained in the maximum wavelength absorption ($\lambda_{max}$) of 516 nm and the linear correlation

coefficient R = 0.9997. To determine the remaining percentage (or residual) DPPH ($DPPH_{rem}$%), equation 1 was used where $DPPH_{t=0}$ is the initial concentration of the DPPH solution and $DPPH_{t=30}$ is the concentration of the reaction mixture with DPPH and sample after 30 min.

$$DPPH_{rem}(\%) = DPPH_{t=30}/DPPH_{t=0} \times 100 \quad (1)$$

The results were presented in terms of half maximal effective concentration ($EC_{50}$) with values in μg $mL^{-1}$, determined from the first-order exponential curve of %$DPPH_{rem}$ *versus* the sample concentrations.[25]

### Antimicrobial activity assay

Strains of *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Candida albicans* (ATCC 90028), and *Candida krusei* (ATCC 6258) were provided by the Biology Laboratory Microorganism Collection of the Federal Institute of Maranhão, Monte Castelo Campus. The medium of culture for tested microorganisms was MH at 37 ºC for 24 h, and they were cultured in SDA at 37 ºC for 48 h before tests. During the experiments, each culture medium was kept at 4 °C.

The minimal inhibitory concentration (MIC) of EECLT, HF, EEF, EAF and EMF were determined using broth dilution method, as recommended by the Clinical Laboratory Standards Institute (CLSI).[26] In this method, 190 μL *per* well of MH or 200 μL *per* well of RPMI-1640 were buffered with 0.165 mol $L^{-1}$ of morpholinepropanesulfonic acid for bacteria or yeasts, respectively, and were added to 96-well microplates.

The samples were dissolved in DMSO and diluted in MH or RPMI-1640 culture medium, depending on which type of tests (bacteria or yeast, respectively). Then, 100 μL of each sample were added to a well of 96-well microplates, and serial dilutions were carried out in subsequently wells in concentrations of 2500 to 4.88 μg $mL^{-1}$. Ciprofloxacin and fluconazole were used as positive controls for bacteria and yeast, respectively.

After that, 10 μL of saline-diluted bacteria inoculum ($1.5 \times 10^8$ CFU $mL^{-1}$) or 100 μL of RPMI-diluted *Candida* inoculum ($1 \times 10^3$ colony forming units (CFU) $mL^{-1}$) were added to each well and incubated at 37 ºC for 24 h in RPMI-1640 medium. After the incubation period, the MIC values were defined as the lowest concentration that visibly inhibited fungal growth. MIC values were confirmed after adding 10 μL of resazurin 0.03% to each well and incubating for 4 h in the dark at 37 ºC. RPMI-1640 or MH medium (100 μL) and standardized inoculum were used as a negative control. DMSO (1% in saline) was also used as a negative control. The results were obtained from three independent assays performed in triplicate.

## Results and Discussion

### Chemical characterization of *C. lanceolatum* by GC-MS, HPLC-ESI-QTOF-MS and DI-ESI-IT-$MS^n$

The investigation by GC-MS analysis of the hexane fraction of *C. lanceolatum* twigs revealed the presence of 28 compounds belonging to different structural types, such as carboxylic acids and derivatives, tocotrienols, terpenoids and an aldehyde and alcohol. The occurrence of terpenoids is frequently observed in the *Combretum* species and other plants in Combretaceae family.[27,28] The total ion chromatogram (TIC) of HF by GC-MS is shown in Figure S1 (Supplementary Information (SI) section).

Saturated (hexadecanoic acid (**3**), octadecanoic acid (**9**)) and polyunsaturated (linoleic acid (**6**)) fatty acids were observed. Their derivatives with methyl (methyl hexadecanoate (**2**)), ethyl (ethyl hexadecanoate (**4**), ethyl octadecanoate (**11**), ethyl eicosanoate (**13**), ethyl oleate (**10**), ethyl linoleate (**8**) and glyceryl (glyceryl hexadecanoate (**15**) and glyceryl monooleate (**16**)) were also identified. The ethyl and methyl derivatives (**2**, **4**, **8**, **10**, **11** and **13**) can be associated to a probable artifacts production in the extraction and the liquid-liquid extraction, respectively, with those solvents being in some cases the responsible to this artifact formation.[29,30] In the literature, fatty acids have demonstrated potential as antibacterial and antifungal agents.[31] All these compounds are been described for the first time in the species.

Eleven terpenoids were identified, including α-cadinol (**1**), kaur-16-ene (**5**), campesterol (**19**), stigmasterol (**20**), sitosterol (**21**), stigmastanol (**22**), β-amyrenone (**23**), methyl commate A (**24**) and stigmast-4-en-3-one (**25**), demonstrated in GC-MS analysis be the main metabolite class in this fraction. They presented a total of relative percentage area of 68.45%, with the compounds **20**, **21** and **24** showing the higher percentage (10.98, 24.5, and 14.66%, respectively). These compounds were also reported in *C. lanceolatum* for the first time. Some of the identified terpenoids presented antibacterial, anticancer, anti-inflammatory, and several others biological applications.[27,32]

Other compounds observed in this species were β-tocopherol (**17**), α-tocopherol (**18**), *cis*-hexadec-9-enal (**7**), δ-octadecalactone, (**12**) and docosanol (**14**). All compounds were determined by comparison with fragments in NIST and WILEY 229 libraries. The structure of identified compounds is shown in Figure 1. Table 1 presented the retention time (min), fragments, molecular

formula, retention index (compared with the literature data) and similarity data for each compound. The mass spectra are disposed in SI section (Figures S2-S26).

The diethyl ether fraction (EEF), ethyl acetate fraction (EAF) and hydromethanolic fraction (HMF) of *C. lanceolatum* twigs were analyzed by

**Table 1.** Identified compounds in hexane fraction of *C. lanceolatum* twigs by GC-MS

| No. | Identification | Molecular mass $[M]^{+\cdot}$ | Molecular formula | Retention time / min | Fragments (*m/z*) base peak in bold | $RI_{exp}$ [a] | $RI_{lit}$ [b] | Relative abundance / % | Similarity / % |
|---|---|---|---|---|---|---|---|---|---|
| **1** | α-cadinol | 222 | $C_{15}H_{26}O$ | 11.262 | 204, 189, 161, 121, **95**, 81, 59, 43 | 1666 | 1652[33] | 0.46 | 84 |
| **2** | methyl hexadecanoate | 270 | $C_{17}H_{34}O_2$ | 18.909 | 227, 143, 87, **74**, 55, 43 | 1927 | 1924[34] | 0.11 | 89 |
| **3** | hexadecanoic acid | 256 | $C_{16}H_{32}O_2$ | 20.165 | 213, 157, 129, 115, 97, 83, **73**, 60, 57, 43 | 1969 | 1964[34] | 9.53 | 96 |
| **4** | ethyl hexadecanoate | 284 | $C_{18}H_{36}O_2$ | 20.974 | 157, 101, **88** | 1997 | 1992[34] | 5.97 | 94 |
| **5** | kaur-16-ene | 272 | $C_{20}H_{32}$ | 22.384 | 257, 229, 213, 187, 175, 161, 147, 125, 105, 91, 81, **69**, **55**, 41 | 2044 | 2042[33] | 0.18 | 81 |
| **6** | linoleic acid | 280 | $C_{18}H_{32}O_2$ | 25.104 | 123, 109, 95, 81, **67**, 55, 41 | 2138 | 2133[33] | 2.15 | 95 |
| **7** | *cis*-hexadec-9-enal | 238 | $C_{16}H_{30}O$ | 25.273 | 135, 123, 111, 98, 69, 55, 41 | 2144 | – | 1.89 | 91 |
| **8** | ethyl linoleate | 308 | $C_{20}H_{36}O_2$ | 25.841 | 263, 178, 164, 150, 135, 109, 95, 81, **67**, 55, 41 | 2164 | 2159[35] | 3.18 | 93 |
| **9** | octadecanoic acid | 284 | $C_{18}H_{36}O_2$ | 25.925 | 241, 185, 171, 143, 129, 97, 83, **73**, 60, **57**, 43 | 2167 | 2161[34] | 0.72 | 91 |
| **10** | ethyl oleate | 310 | $C_{20}H_{38}O_2$ | 26.015 | 264, 123, 111, 98, 83, 69, **55**, 43, 41 | 2170 | 2179[35] | 2.35 | 85 |
| **11** | ethyl octadecanoate | 312 | $C_{19}H_{38}O_2$ | 26.774 | 157, 101, **88**, 70, 55, 43, 41 | 2196 | 2192[34] | 1.73 | 91 |
| **12** | δ-octadecalactone | 282 | $C_{18}H_{34}O_2$ | 31.100 | **99**, 83, 70, 55, 43, 41 | 2354 | – | 0.17 | 82 |
| **13** | ethyl eicosanoate | 340 | $C_{22}H_{44}O_2$ | 32.231 | 297, 157, 143, 101, **83**, 73, 57, 55, 43 | 2397 | 2395[36] | 0.59 | 88 |
| **14** | docosanol | 410 | $C_{22}H_{46}O$ | 34.687 | 139, 125, 111, 97, 83, 69, **57**, 55, 43, 41 | 2492 | 2500[37] | 0.23 | 91 |
| **15** | glyceryl hexadecanoate | 330 | $C_{19}H_{38}O_4$ | 35.130 | 257, 239, 134, 112, **98**, 84, 74, 69, 57, 55, 43, 41 | 2506 | 2502[38] | 0.99 | 92 |
| **16** | glyceryl oleate | 354 | $C_{21}H_{38}O_4$ | 39.497 | 262, 149, 135, 121, 109, 95, 81, **67**, 55, 41 | 2688 | 2684[38] | 0.85 | 87 |
| **17** | β-tocopherol | 416 | $C_{28}H_{48}O_2$ | 47.688 | 207, 191, **151** | 3056 | 3047[38] | 0.44 | 82 |
| **18** | α-tocopherol | 430 | $C_{29}H_{50}O_2$ | 49.397 | 205, **165** | 3138 | 3142[34] | 0.47 | 85 |
| **19** | campesterol | 400 | $C_{28}H_{48}O$ | 51.209 | 382, 367, 315, 289, 255, 231, 213, 145, 119, 107, 95, 81, 55, **43** | 3228 | 3243[34] | 1.45 | 83 |
| **20** | stigmasterol | 412 | $C_{29}H_{48}O$ | 51.921 | 300, 271, 255, 213, 159, 145, 133, 105, 97, 83, 69, **55**, 43, 41 | 3264 | 3242[8] | 10.98 | 93 |
| **21** | sitosterol | 414 | $C_{29}H_{50}O$ | 53.158 | 396, 381, 329, 303, 255, 213, 159, 145, 133, 119, 107, 95, 81, 69, 57, **43** | 3327 | 3332[39] | 24.50 | 90 |
| **22** | stigmastanol | 416 | $C_{29}H_{52}O$ | 53.280 | 401, 233, **215**, 165, 147, 135, 121, 107, 95, 81, 69, 55, **43** | 3333 | – | 1.02 | 85 |
| **23** | β-amyrenone | 424 | $C_{30}H_{48}O$ | 53.708 | **218**, 203, 189, 135, 109, 95, 81, 69, 55, 43 | 3356 | – | 9.15 | 89 |
| **24** | methyl commate A | 500 | $C_{32}H_{52}O_4$ | 54.615 | **218**, 203, 189, 135, 109, 95, 81, 69, 55, 43 | 3403 | – | 14.66 | 87 |
| **25** | stigmast-4-en-3-one | 412 | $C_{29}H_{48}O$ | 55.661 | 370, 289, 229, 147, **124**, 109, 95, 55 | 3459 | – | 2.77 | 86 |

[a]Experimental retention index; [b]retention index from literature (compared with similar columns analysis). *m/z*: mass-to-charge ratio; $[M]^{+\cdot}$: ionized molecular mass with an electron loss.

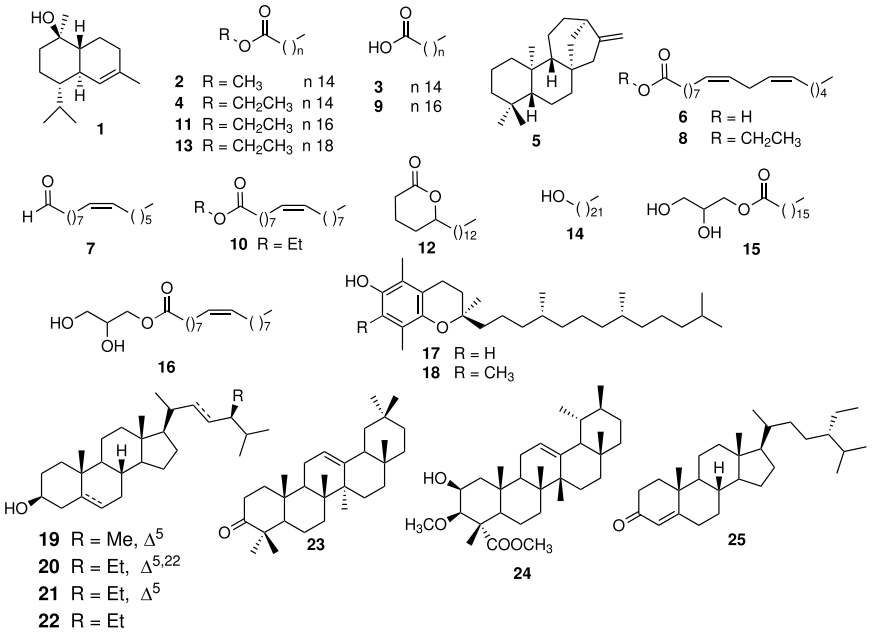


**Figure 1.** Structures of identified compounds in hexane fraction of *C. lanceolatum* twigs.

HPLC-ESI-qTOF-MS/MS and DI-ESI-IT-MS$^n$ in negative ion mode, to obtain a better chemical profile of the fractions. Using these techniques, different gallic acid derivatives, including galloyl esters of glucose (gallotannins) and some hexahydrodiphenoyl (HHDP) esters of glucose (ellagitannins), phenolic compounds (such as precursor acids) and flavonoids (aglicon and/or glycosylated derivatives) were identified.

A total of thirty-two compounds (**26**-**57**) were observed, with the EAF presenting the largest number of identified compounds (twenty-nine). The high-resolution molecular mass, error (ppm), fragments (mainly in low resolution) and the presence in each fraction of the identified compounds are described in Table 2. The total ion chromatogram (TIC) for each analyzed fraction is shown in Figure S27 and the structural proposition for each compound is shown in Figure 2.

**Table 2.** High-resolution and low-resolution fragments and identification of diethyl ether fraction (EEF), ethyl acetate fraction (EAF) and hydromethanolic fraction (HMF) of *C. lanceolatum* twigs by DI-ESI-IT-MS$^n$ and HPLC-ESI-QTOF-MS/MS in negative ion mode

| No. | Compound | $t_R$ / min | HR-MS $[M - H]^-$ | Error / ppm | LR-MS $[M - H]^-$ | MS/MS$^n$ (percentage abundance / %) | EEF | EAF | HMF | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| **26** | malic acid | nd | nd | nc | 133 | 114.8(100) | – | + | – | 40 |
| **27** | galic acid | nd | nd | nc | 169 | 124.9(100) | + | + | – | 40, 41 |
| **28** | methyl gallate | nd | nd | nc | 183 | 180.7(32), 167.8(100), 123.8(49) | – | + | – | 40, 42 |
| **29** | (epi)-catechin | nd | nd | nc | 289 | 244.8(100), 230.8(15), 204.8(47), 178.8(14), 136.9(9), 124.8(6) | + | + | – | 40, 43 |
| **30** | brevifolin carboxylic acid | nd | nd | nc | 291 | 246.8(100), 204.8(34) | – | + | – | 40 |
| **31** | ellagic acid | 7.394 | 301.0005 | 5.0 | 301 | 272.8(30), 256.8(100), 228.8(64) | + | + | – | 44 |
| **32** | quercetin | nd | nd | nc | 301 | 238.2(15), 178.8(86), 150.8(84) | + | + | – | 40 |
| **33** | (epi)-gallocatechin | 2.908 | 305.0674 | 2.6 | 305 | 286.9(10), 272.8(20), 260.9(25), 246.8(18), 224.9(34), 220.9(78), 218.9(85), 178.8(100), 164.8(29), 136.8(24), 124.8(42) | – | + | + | 40, 45 |
| **34** | 3'-*O*-methyl-quercetin | nd | nd | nc | 315 | 299.8(87) | – | + | – | 40 |
| **35** | protocatechuic acid | nd | nd | nc | 315 | 152.8(100) | – | + | – | 40 |
| **36** | dimethyl-ellagic acid | 9.455 | 329.0313 | 3.0 | nd | 314.0116(54), 298.9843(100), 270.9749(24) | + | – | – | 46 |
| **37** | galloyl-*O*-hexoside | nd | nd | nc | 331 | 168.7(100) | – | + | – | 47, 48 |
| **38** | trimethyl-ellagic acid | 10.652 | 343.0486 | 2.9 | nd | 312.9891(100) | + | – | – | 46 |

**Table 2.** High-resolution and low-resolution fragments and identification of diethyl ether fraction (EEF), ethyl acetate fraction (EAF) and hydromethanolic fraction (HMF) of *C. lanceolatum* twigs by DI-ESI-IT-MS$^n$ and HPLC-ESI-QTOF-MS/MS in negative ion mode (cont.)

| No. | Compound | $t_R$ / min | HR-MS $[M - H]^-$ | Error / ppm | LR-MS $[M - H]^-$ | MS/MS$^n$ (percentage abundance / %) | EEF | EAF | HMF | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| **39** | ellagic acid-*O*-pentoside | 6.942 | 433.0455 | 10.6 | 433 | MS2: 300.8(100), 299.8(98) MS3(301): 299.0(100), 256.7(42), 184.8(46) | + | + | – | 49 |
| **40** | ellagic acid-*O*-deoxyhexoside | nd | nd | nc | 447 | MS2: 300.9(100) MS3(301): 272.8(29), 254.8(42) | – | + | – | 50 |
| **41** | quercetin-*O*-deoxy hexoside | 7.857 | 447.0981 | 10.7 | 447 | MS2: 300.9(100) MS3(301): 178.7(95), 150.8(100) | + | + | – | 40 |
| **42** | ellagic acid-*O*-hexoside | nd | nd | nc | 463 | MS2: 300.8(100) MS3(301): 270.9(75), 254.8(46) | + | + | – | 50 |
| **43** | quercetin-*O*-hexoside | 7.073 | 463.0934 | 11.2 | 463 | MS2: 300.8(100) MS3(301): 178.7(100), 150.8(67) | + | + | – | 40 |
| **44** | valoneic acid lactone | 2.930 | 469.0934 | 5.3 | 469 | 424.8(100) | + | + | – | 40 |
| **45** | miricetin-*O*-hexoside | nd | nd | nc | 479 | 461.0(15), 316.9(100), 296.8(84), 270.8(6), 178.8(6), 166.8(15) | – | + | – | 40 |
| **46** | HHDP-hexoside | 1.590 | 481.0608 | 3.3 | 481 | 444.9(11), 312.8(27), 300.8(100), 274.8(14), 168.8(7) | + | + | + | 40, 41 |
| **47** | digalloyl-hexoside | 3.812 | 483.0814 | 7.0 | 483 | 450.8(21), 438.9(40), 330.9(20), 312.8(65), 301.8(100), 270.8(48), 210.8(17), 168.8(33) | – | + | – | 51 |
| **48** | methyl-galloyl-galloyl-hexoside | nd | nd | nc | 497 | 481.9(17), 461.0(35), 450.9(75), 340.9(15), 326.9(72), 312.9(100), 182.8(43), 168.8(54) | – | + | – | 51 |
| **49** | (epi)-catechin-(epi)-catechin | 3.907 | 577.1357 | 0.9 | 577 | 560.9(41), 450.9(27), 424.9(94), 406.9(100), 288.9(37) | – | + | + | 40, 52 |
| **50** | (epi)catechin-(epi)-gallocatechin | 2.257 | 593.1305 | 0.7 | 593 | 574.9(28), 466.9(38), 440.9(39), 422.9(100), 304.9(44) | – | + | + | 40, 52 |
| **51** | galloyl-HHDP-hexoside | 2.759 | 633.0780 | 4.3 | 633 | 541.8(6), 480.9(6), 300.8(100), 274.8(17), 256.8(6), 228.8(4) | + | + | – | 40, 41 |
| **52** | bis-HHDP-hexoside | 2.400 | 783.0709 | 2.9 | 783 | 601.8(11), 541.3(12), 480.9(16), 300.8(100), 274.8(15) | + | + | – | 40, 41 |
| **53** | (epi)catechin-(epi)-catechin-(epi)-catechin | 3.146 | 865.1999 | 1.6 | 865 | 847.1(15), 739.0(43), 695.0(100), 576.4(62), 406.9(58), 286.9(51) | – | + | – | 40, 52 |
| **54** | galloyl-bis-HHDP-hexoside | nd | nd | nc | 935 | 781.5(33), 721.9(55), 632.9(47), 601.8(100), 463,0 (20), 450.8(35), 300.8(23) | – | + | – | 40, 41 |
| **55** | chebulagic acid | nd | nd | nc | 953 | 906.9(100), 781.0(42), 601,9 (14), 503.1(28), 300.9(20) | – | + | – | 53 |
| **56** | punicalagin | 2.003 | 1083.0585 | 0.7 | 1083 | 1064.9(10), 780.9(30), 600.9(100), 574.9(19), 448.9(14), 392.8(10), 298.8(5) | + | + | + | 40, 44 |
| **57** | galloylpunicalagin | nd | nd | nc | 1235 | 1082.8(83), 600.8(100) | – | + | – | 40, 44 |

$t_R$: retention time in HPLC-ESI-TOF-MS analysis; HR-MS: high resolution mass by HPLC-ESI-TOF-MS; LR-MS: low resolution mass by DI-ESI-IT-MS$^n$; $[M - H]^-$: deprotonated molecular ion; MS/MS$^n$: product ions; ppm: parts *per* million; nd: not done; nc: not calculated; – not present; + present; HHDP: hexahydrodiphenoyl.

The compound **26**, with ion at *m/z* 133 $[M - H]^-$, produced a fragment ion at *m/z* 115 $[M - 18 - H]^-$ by a neutral loss of water ($H_2O$). It was identified as malic acid (**26**).[40] The precursor ion at *m/z* 169 $[M - H]^-$ yield a product ion at *m/z* 125 by neutral loss of a $CO_2$ (–44 Da), identified as gallic acid (**27**), a compound usually found in plants.[41] A derivative of compound **27** with ion at *m/z* 183 $[M - H]^-$, presented fragments ions at *m/z* 169 and 125 by loses of a methyl (–15 Da) and $CO_2$ (–44 Da), consecutively, and it was identified as methyl gallate (**28**).[42]

A total of eight flavonoids or their glycosylated derivatives were identified. Their fragmentation pathways tended to loses of neutral CO, $H_2O$ and methyl groups (28, 18 and 15 Da, respectively), and other important fragments

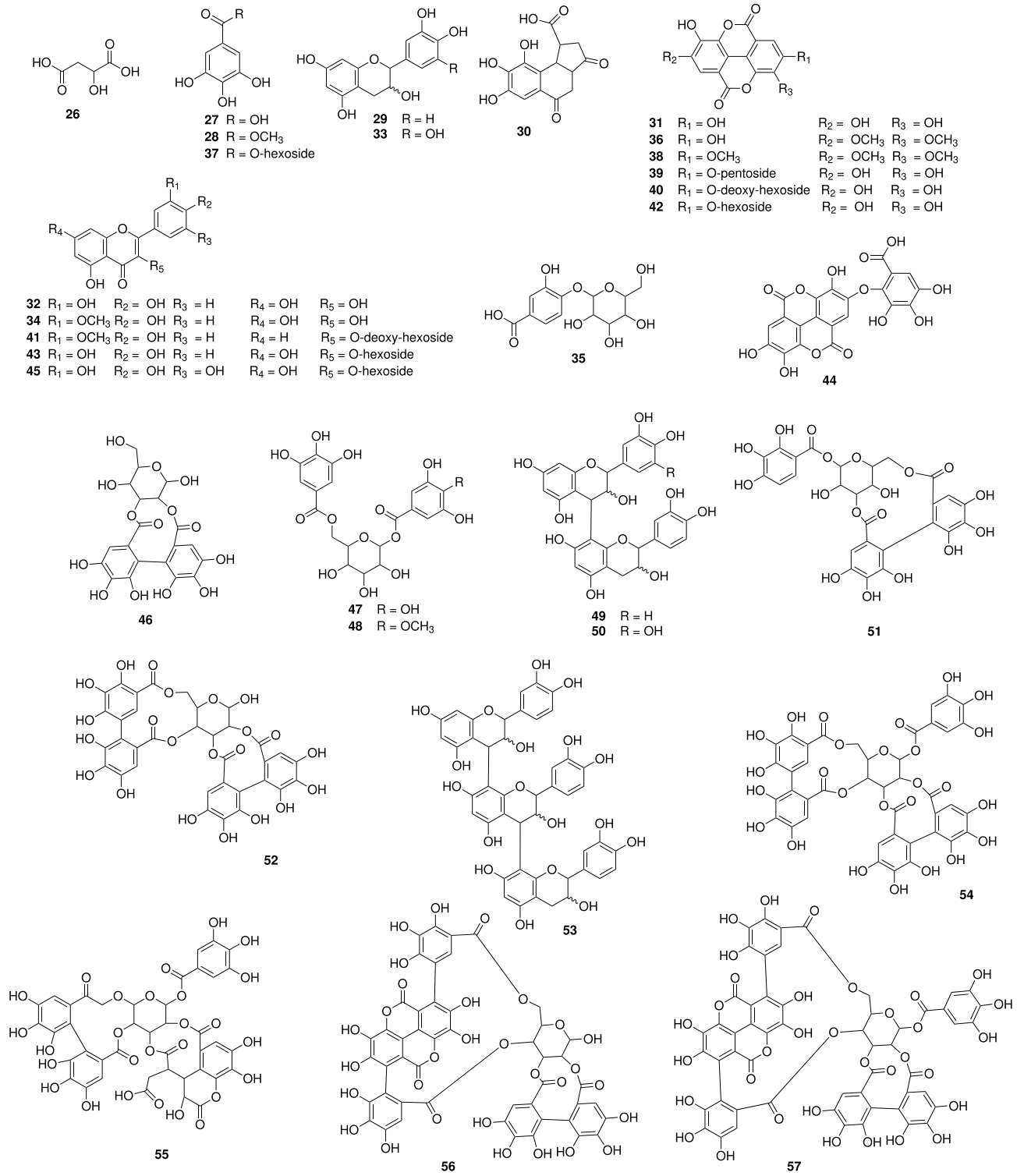


**Figure 2.** Structures of identified compounds in ethyl ether, ethyl acetate and hydromethanolic fractions of *C. lanceolatum* twigs by DI-ESI-IT-MS$^n$ and/or HPLC-ESI-TOF-MS/MS.

observed with the cleavages of rings A/C, characteristic of retro-Diels-Alder (RDA) fragment, and C/B, typical of the entire loss of ring B.[54] The fragmentation of compounds depends of each group and variates with their position in structures.

The compound **29** presented a deprotonated ion at *m/z* 289 $[M - H]^-$ showed as the main fragment an ion at *m/z* 245 $[M - 44 - H]^-$ by the loss of a $CO_2$ molecule. Cleavages of RDA reaction results in a fragment at *m/z* 137 $[M–152–H]^-$, with another cleavage in C ring that

generate an ion at *m/z* 125 [M – 164 – H]$^-$. An entire loss of B ring produced an ion at *m/z* 179 [M – 110 –H]$^-$. It was identified as (epi)-catechin (**29**), widely distributed in plants.[40,43] Compound **33** is a mono-hydroxylated derivative of catechin. The compound detected at *m/z* 305 [M – H]$^-$ presented the same fragment ions of compound **29**, but it showed a different fragment by a loss of $CO_2$ molecule at *m/z* 261 [M – 44 – H]$^-$ confirming the hydroxyl group in B ring. It was identified as (epi)-gallocatechin (**33**).[40,45]

Compound **32** showed a deprotonated ion at *m/z* 301[M – H]$^-$, with the characteristic fragments of flavonoids, at *m/z* 179 [M – 122 – H]$^-$ and *m/z* 151 [M – 150 – H]$^-$, identified as quercetin (**32**).[40] Other compound with ions at *m/z* 315 [M – H]$^-$ presented similar structure with the difference of 14 Da, with addition of one methyl group. The fragmentation pattern presented loss of 15 Da, the methyl group [M – 15 – H]$^-$ and it was identified as 3'-*O*-methyl-quercetin (**34**).[40]

The fragmentation pattern of glycosylated flavonoids (compounds **41**, **43** and **45**) produced ions of the respective aglycones, with loses of hexoses (–162 Da) or deoxy-hexoses (–146 Da). The MS$^2$ spectrum of ion at *m/z* 447 [M – H]$^-$ presented a base peak fragment at *m/z* 301 [M – 146 – H]$^-$, characteristic of a deoxy-hexose loss, and ions at *m/z* 179 [M – 268 – H]$^-$ and 151 [M – 296 – H]$^-$ attributed to quercetin, this compound was identified as quercetin-*O*-deoxy-hexoside (**41**).[40] The MS$^2$ spectrum of the precursor ion at *m/z* 463 [M – H]$^-$ provided the fragment ions at *m/z* 301 [M – 162 – H]$^-$ indicating loss of a hexose unit. The MS$^3$ (*m/z* 463 → 301) presented fragment ions at *m/z* 255 [M – 208 – H]$^-$, 179 [M – 284 – H]$^-$ and 151 [M – 312 – H]$^-$ and it was identified as quercetin-*O*-hexoside (**43**). The ion at *m/z* 479 [M – H]$^-$ showed a base peak at *m/z* 317 [M – 162 – H]$^-$ indicating also a hexose loss unit. Other ions at *m/z* 297 [M – 182 – H]$^-$ and 179 [M – 312 – H]$^-$made it possible, compared to the literature, the identification as myricetin-*O*-hexoside (**45**).[40]

Compound **31** was identified as ellagic acid and presented a deprotonated ion at *m/z* 301 [M – H]$^-$ and a fragment ion at *m/z* 257 [M – 44 – H]$^-$ resulting from the typical loss of a $CO_2$.[44] Glycosylated derivatives were observed with molecular ion at *m/z* 433 [M – H]$^-$, 447 [M – H]$^-$ and 463 [M – H]$^-$. The MS$^2$ spectrum of the precursor ion provided the fragment ions at *m/z* 301 [M – 132 – H]$^-$, [M – 146 – H]$^-$and [M – 162 – H]$^-$, respectively, representing the loss sugar units of pentose, deoxy-hexose and hexose. The MS$^3$ (*m/z* 433 → 301) presented fragment ions at *m/z* 299 [M – 134 – H]$^-$, 257 [M – 132 – 44 – H]$^-$, and 185 [M – 132 – 116 – H]$^-$. These compounds were identified as ellagic acid-*O*-pentoside (**39**), ellagic acid-*O*-deoxy-hexoside (**40**) and ellagic acid-*O*-hexoside (**42**).[49,50]

Three procyanidins were identified with ions at *m/z* 577 [M – H]$^-$, 593 [M – H]$^-$ and 865 [M – H]$^-$ for compounds **49**, **50** and **53**, respectively. The fragment ions at *m/z* 289, 305 and 287, were observed for (epi)-catechin and (epi)-gallocatechin, and the ions at *m/z* 407, 423, 425, 441 and 695 for the retro-Diels-Alder (RDA) fragments. The compounds and fragmentation pattern were already described in the literature and have been identified as (epi)catechin-(epi) catechin (**49**), (epi)catechin-(epi)gallocatechin (**50**) and (epi)catechin-(epi)catechin-(epi)catechin (**53**).[40,52]

The MS$^2$ spectrum fragments of compounds **46**, **51**, **52** and **54** with deprotonated ions at *m/z* 481 [M – H]$^-$, 633 [M – H]$^-$, 783 [M – H]$^-$ and 935 [M – H]$^-$, respectively, showed the presence of a product ion at *m/z* 301, attributed to an ellagic acid fragment, resultant from the intramolecular lactonization of each carboxyl group of the HHDP unit with two hydroxyl groups of the same HHDP unit. These data results in identification of HHDP-hexoside (**46**), galloyl-HHDP-hexoside (**51**), bis-HHDP-hexoside (**52**) and galloyl-bis-HHDP-hexoside (**54**).[40,41]

Four compounds were identified as esters of gallic acid and polyol, usually hexoses. These gallotannins showed the characteristic fragment ions in their product ion spectra by consecutive elimination of galloyl and gallate moieties. Compound **37** with ion at *m/z* 331 [M – H]$^-$ produced a fragment at *m/z* 169 [M – 162 – H]$^-$, by a loss of hexose, as the deprotonated gallic acid ion as the main fragment, corresponding to galloyl-*O*-hexoside (**37**).[47,48] The compounds **47** and **48** with ions at *m/z* 483 [M – H]$^-$ and 497 [M – H]$^-$, both compounds provided fragment ions at *m/z* 313 [M – 184 – H]$^-$ and 169 [M – 162 – H]$^-$ determined as a galloyl-hexoside group and galloyl moiety, identified as digalloyl-hexoside (**47**) and methylgalloyl-galloyl hexoside (**48**), respectively.[51] The mass spectrum of compound **55** presented a deprotonated ion at *m/z* 953 [M – H]$^-$ and fragment ions at *m/z* 907 [M – 46 – H]$^-$ and 301 [M –652 – H]$^-$characteristic of loss of a formyl acid and the formation of HHDP ion unit, respectively. This compound was identified as chebulagic acid (**55**).[53]

The compound **56** with ion at *m/z* 1083 [M – H]$^-$, showed fragments at *m/z* 781 [M – 302 – H]$^-$ and 601 [M – 634 – H]$^-$ corresponding to gallagyl-hexosyl and gallagyl units, respectively, previously described in the literature as pungicalagin (**56**).[40,44] The compound **57** with ion at *m/z* 1235 [M – H]$^-$ presented fragments ions at *m/z* 1083 [M – 152 – H]$^-$ and 601 [M – 634 – H]$^-$ attributed to losses of gallic acid and the formation of gallagyl fragment, respectively. This compound was identified as galloylpunicalagin (**57**).[40,44]

Other three phenolic compounds **30**, **35** and **44** were identified and showed deprotonated ion at *m/z* 291 $[M - H]^-$, 315 $[M - H]^-$ and 469 $[M - H]^-$, respectively. The precursor ion of each compound produced fragment ions by neutral loss of a $CO_2$ group (–44 Da), attributed to phenolic compounds. The compound **35** presented, in addition to this $CO_2$ loss, an ion at *m/z* 153 $[M - 162 - H]^-$ characteristic of a hexose loss. These compounds were identified as brevifolin carboxylic acid (**30**), protocatechuic acid (**35**) and valoneic acid dilactone (**44**).[40]

Unlike DI-ESI-IT-$MS^n$, the HPLC-QTOF-MS/MS did not present many information about the fragmentation of the analyzed fractions, resulting as the main information the high-resolution mass, which leads to the possibility of the error calculation to confirm the molecular formula of each peak. Not all identified compounds by DI-ESI-IT-$MS^n$ were detected in HPLC-ESI-QTOF-MS/MS, and two compounds were identified only by this high-resolution technique. The compounds **36** and **38** presented molecular ion at *m/z* 329.0313 $[M - H]^-$ and 343.0486 $[M - H]^-$, respectively, and showed fragment ions at *m/z* 314.0116 $[M - 15 - H]^-$, 298.9843 $[M - 30 - H]^-$ and 270.9749 $[M - 58 - H]^-$ for compound **36** and a fragment at *m/z* 312.9891 $[M - 30 - H]^-$ for compound **38**, characteristic of a methyl losses and $CO_2$ unit. These compounds (**36** and **38**) presented two and three additional methyl groups, respectively, and were identified as dimethyl-ellagic acid (**36**) and trimethyl ellagic acid (**38**).[46] The mass spectrum for each identified compound with $MS^2$ and $MS^3$ (when observed) by DI-ESI-IT-$MS^n$ and/or HPLC-ESI-QTOF-MS/MS are disposed in Figures S28 to S59 (SI section).

All these compounds (**26** to **57**) were mentioned previously within the Combretaceae family. However, this study reveals their presence for the first time in *C. lanceolatum* species, with the exception of compounds **32**, **34** and **41**, already described in this species. This shows a large number of different phenolic compounds, such as gallotannins, ellagitanins and flavonoids, in *C. lanceolatum* twigs.

### Total phenolic content and antioxidant activity

The TPC was calculated for the EECLT and showed a value of 583.2 ± 39 expressed in gallic acid equivalent milligrams *per* extract grams (GAE $g^{-1}$). In comparation with the literature data, the EECLT demonstrated a high amount of phenolic,[55,56] and from this result, the EECLT was tested for its antioxidant capability. Also, the polar fractions (EEF, EAF and HMF) of which were already identified some phenolic compounds were evaluated, since these metabolites can be the main responsible for this activity.[56]

The evaluation of the antioxidant activity of the extract EECLT and EEF, EAF and HMF fractions was developed by the DPPH assay method, of which displayed the antiradical capacity of the sample.[25] The percentage of antioxidant capability for all tested concentrations and half medium efficient concentration ($EC_{50}$) of the extract and fractions were given in Table 3.

The EECLT and EAF showed low values of $EC_{50}$ (57.9 ± 5.5 and 45.4 ± 0.6, respectively) and the EEF and HMF could not be determinate. The EECLT and EAF showed a promising antioxidant activity, better than the synthetic compound BHT ($EC_{50}$ 69.34 ± 5.53), which is already described in the literature as a good antioxidant agent.[57]

These results can demonstrate that compounds presented in EECLT and EAF (the fraction with the largest number of phenolic compounds identified) may act as a donor of hydrogen radicals which can stabilize the DPPH radical forming hydrazine. As already described in the literature, flavonoids and phenolic compounds of plant extracts are responsible for antioxidant properties, since they contain OH-groups in resonance on phenolic groups,

**Table 3.** Antioxidant and antimicrobial activities of ethanolic extract and its fractions from *C. lanceolatum* twigs

| Sample | Antioxidant activity $EC_{50}$ / (μg $mL^{-1}$) | Antimicrobial activity MIC / (μg $mL^{-1}$) | | | |
|---|---|---|---|---|---|
| | | *S. aureus* | *E. coli* | *C. albicans* | *C. krusei* |
| EECLT | 57.91 ± 5.54 | 125 | 62.5 | 3.9 | 15.6 |
| HF | nd | 250 | 250 | 250 | 250 |
| EEF | nc | nd | 62.5 | nd | nd |
| EAF | 45.39 ± 0.60 | 250 | 500 | 125 | 125 |
| HMF | nc | 250 | 500 | 125 | 125 |
| BHT[a] | 69.34 ± 5.53 | – | – | – | – |
| Ciprofloxacin[b] | – | 0.5 | 0.0625 | – | – |
| Fluconazole[c] | – | – | – | 8 | 16 |

[a]Positive control for antioxidant activity; [b]positive control for antibacterial activity; [c]positive control for antifungal activity. $EC_{50}$: half maximal effective concentration; MIC: minimum inhibitory concentration; nd: not done; nc: not calculated.

characterized by the stabilization of the radical by the electron delocalization.[58,59]

The presence in EAF of the compounds such as quercetin (**32**), ellagic acid (**31**), gallic acid (**27**) and (epi)-catechin (**29**) with antioxidant potential already described in the literature and their respective derivatives demonstrated and reinforces the good antioxidant potential of the respective fraction and also for the extract.[60-63]

These data are compatible with the data reported for the extracts from other species of the Combretaceae family such as *Terminalia brasiliensis* (bark and leaf) and *C. quadrangulare*, demonstrating the antioxidant potential for Combretaceae family and *Combretum* genus.[7,25]

### Antimicrobial activity

In antibacterial assay evaluated for *S. aureus* and *E. coli*, of EECLT and HF, EEF, EAF and HMF, the samples presented values of MIC (μg $mL^{-1}$) in range of 62.5 to 125 for EECLT, 250 for HF, 62.5 for EEF, 250 to 500 for EAF and HMF and 0.0625 to 0.5 for the positive control ciprofloxacin (CPR). The EECLT exhibited a lower value of MIC than the fractions, demonstrating the best result compared to all analyzed samples, with a good and moderate potential against *S. aureus* and *E. coli*, respectively. As described in the literature, values of MIC less than 100 μg $mL^{-1}$ are considered good, from 100 to 500 μg $mL^{-1}$ moderate, from 500 to 1000 μg $mL^{-1}$ weak and over 1000 μg $mL^{-1}$ are considered inactive.[64] Table 3 shows the values of MIC (μg $mL^{-1}$) evaluated for the EECLT and for the fractions HF, EEF, EAF and EMF against the strains of *S. aureus* (ATCC 29213) and *E. coli* (ATCC 25922).

The antifungal potential against strains of *C. albicans* and *C. krusei* were also shown in Table 3. The values of MIC (μg $mL^{-1}$) were 3.9 and 15.6 for EECLT, 250 for HF and 125 for EAF and HMF, with the positive control (fluconazole) presenting values of 8 and 16, respectively. The antifungal potential for EECLT, with results better than FLZ, can be associated to a possible synergistic association of fatty acids, triterpenoids and phenolic substances (flavonoids and tannins), since they had already shown this activity in the literature.[31,65-67]

All tested samples were considered active, with the extract showing a good potential in application for antifungal effects against yeasts of *Candida* genus, with MIC values lower than the drug used in treatment of this fungus. There are no reports in literature on the antimicrobial activity of *C. lanceolatum* twigs, but there are some reports of antimicrobial potential for others *Combretum* species.[6,68]

## Conclusions

The study of fractions from *C. lanceolatum* twigs led to the identification of fifty-seven compounds: such as terpenoids, fatty acids, tocopherols, gallotannins, ellagitannins, flavonoids and other phenolic compounds. The EECLT and EAF presented antioxidant activity, by DPPH assay, which can be associated with the great number of phenolic compounds in these samples and with more studies, could be used as antioxidant complementary source. The extract and all fractions presented promising results for antibacterial activity with the EECLT presenting the best values of minimal inhibitory concentration (MIC) against *S. aureus* and *E. coli*. In antifungal activity, the EECLT shown better values than the positive control fluconazole against both *Candida* species, although they need more studies to address which type of metabolite can be responsible for this antimicrobial potential and for a later application against *Candida* infection. This study presents relevant chemical and biological information about the *C. lanceloatum* species expanding the knowledge about the flora of the northeast of Brazil.

## Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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### Author Contributions

Evaldo S. Monção Filho was responsible for investigation, methodology, writing original draft, visualization and writing review and editing; Mariana Helena Chaves for resources and supervision; Ruth Raquel S. Farias for investigation; João Sammy N. Souza for resources and supervision; Carmem D. L. Campos for investigation; Cristina A. Monteiro for investigation and supervision; Éverton Leandro F. Ferreira

for investigation; Mônica T. Pupo for investigation and supervision; Gerardo M. Vieira Júnior for resources, supervision, conceptualization, project administration and writing review and editing.

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