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Article

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF EXTRACTS AND ISOLATED COMPOUNDS FROM THE ROOTS OF *Bowdichia virgilioides* (Fabaceae)

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This study describes the chemical composition and biological activities of extracts and compounds obtained from the roots of *Bowdichia virgilioides* (Fabaceae). A mixture of very long-chain free fatty acids (VLFAs) and pure compounds, namely isocordoin, cordoin, lupeol, lupenone, β-sitosterol, stigmasterol, and sitostenone, were isolated from the soluble fraction of the methanol extract by using sequential column chromatography procedures. The molecular structures of the compounds were determined using infrared (IR), nuclear magnetic resonance (NMR), and mass spectrometry (MS) techniques. Notably, this is the first report of the occurrence of both chalcones within this plant genus. Besides, the VLFAs were identified by electrospray ionization mass spectrometry (ESIMS) and the presence of these compounds in roots is suggested to be an adaptive response to environmental stress, which exhibited moderate antioxidant properties when tested against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and in the β-carotene/linolenic acid auto-oxidation assay. However, the MeOH soluble fraction demonstrated significant toxicity in the brine shrimp lethality assay.

Keywords: Fabaceae; *Bowdichia virgilioides*; triterpenes; chalcones; brine shrimp test; antioxidant activities.

INTRODUCTION

Fabaceae is one of the most diverse and abundant plant families, with approximately 770 genera and about 19,500 species, ranking the third major among Angiosperms. The family comprises three subfamilies: Mimosoideae, Faboideae, and Caesalpinioideae, each with distinctive morphological and molecular features.[1]

Bowdichia virgilioides Kunth. is a tree with various local names such as "sucupira-branca", "sucupira-verdadeira" or "sucupira-preta". It belongs to the subfamily Faboideae and grows in the tropical forests of South America. It has economic value because its wood suits carpentry and woodcrafts.[2] In traditional medicine, especially in the northeast of Brazil, the roots are used for rheumatism; the barks for ulcers, diabetes, and diarrhea; the seeds for fever, inflammation, gout, and arthritis; the leaves for sore throat and joint pain;[3] while the seeds are used in the treatment of rheumatism, arthritis, and skin diseases.[4] The aqueous extracts of *B. virgilioides* have antinociceptive,[5] anti-inflammatory, and analgesic effects.[6] A recent study[7] identified a compound called 8-methoxy coumestrol and a *trans* derivative of the *p*-coumaric ester, isolated from the stem of *B. virgilioides*, that inhibited cathepsin V. Cathepsin V is a potential marker for colon cancer diagnosis.[8] Previous investigation on the chemical constitution of this species reports the isolation of flavonoids,[9] isoflavonoids,[10] triterpenes, and diaza-adamantane alkaloids.[11,12]

However, due to the similar folk name, this plant is often confused with the those of *Pterodon pubescens* (Benth.) Benth fruits.[13] *Pterodon* spp. is known to produce vouacapanes diterpenoids. Vouacapanes represent an important group of tetracyclic cassanes diterpenes and their structure is characterized by a tricyclic skeleton and one furan ring, and these compounds are known to present analgesic *in vivo* activities. On the other hand, *B. virgilioides* does not present this class of diterpenes.[14]

Despite the importance of this species as folk medicine, studies on its chemical composition are still scarce. As a contribution, this work describes the isolation and characterization of the chemical constituents and the evaluation of the antioxidant and cytotoxic potential of the extracts, using Artemia salina toxicity, from the roots of *B. virgilioides*.

EXPERIMENTAL

General procedures

Si gel 60 F254 of 0.25 and 1 mm thickness from Merck (Darmstadt, Germany) was employed in thin layer chromatography (TLC) plates. The elution was accomplished by UV cabinet (254 and 336 nm), iodine vapors, $FeCl_3$ solution, or Liebermann-Burchard reagent. The conventional column chromatography (CC) separations were carried out with silica gel 60 (0.063-0.200 nm) and silica flash (0.040-0.063 mm) from Acros (Antwerpen, Belgium) and Sephadex LH-20 from Pharmacia (Milan, Italy). All PA solvents used (hexane, MeOH, $CHCl_3$, EtOAc, dichloromethane (DCM)) in chromatography and general procedures were from Quimex (Uberaba, Brazil) and Tedia (Rio de Janeiro, Brazil). Infrared (IR) spectra were obtained with an IRAffinity-1 spectrophotometer (Shimadzu, Kyoto, Japan) employing dry KBr plates or pellets. 1D and 2D nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AvanceIII-500 MHz spectrometer (Bruker, Rheinstetten, Germany) using traditional pulse sequences for the homonuclear and heteronuclear correlation experiments, and methanol-d_4 (Cambrige, Tewksbury, Massachusetts, USA) as a solvent. Chemical shifts (δ) were referenced to the residual peaks of the deuterated solvent in relation with tetramethylsilane (TMS), acquired from Central Analítica from Universidade de São Paulo (São Paulo, Brazil).

Gas chromatography-mass spectrometry (GC-MS) analyses were performed in a Shimadzu mod. QP2010SE equipment (Kyoto, Japan). The injector was set at 290 °C, and the analysis was run with an initial temperature of 50 °C min^{-1}, ranging from 50 to 180 °C at a ratio of 15 °C min^{-1}, 180 to 230 °C at a ratio of 7 °C min^{-1}, and from 230 to 250 °C at a ratio of 15 °C min^{-1}, remaining at this temperature for 10 min. Subsequently, the temperature rose to 285 °C at the 4 °C min^{-1} rate, thus remaining for 40 min. The detector temperature and the

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temperature at the interface of the GC-MS system was maintained at 290 °C. The mass registration was carried out by electron impact ionization (70 eV) and by scanning masses between 35 and 1000 Da. The compounds were identified by comparing the samples' mass spectra with databases (NIST21, NIST107, and WILEY229) and a fatty acid methyl esters (FAME) standard (Aldrich). The electrospray ionization mass spectrometry (ESIMS) analysis was carried out in an equipment mod. Amazon speed ETD from Bruker Daltonics (Rheinstetten, Germany) of Central Analítica of Instituto de Química (USP) and registered in positive and negative mode.

Plant material

The roots of *B. virgilioides* were collected in the campus Ondina of the Universidade Federal da Bahia (12°59'56.148"S, 38°30'29.016"W). Prof. Dr. Maria L. S. Guedes identified the species, and the sample was deposited in the Alexandre Leal Costa Herbarium of the Institute of Biology of UFBA (voucher No. 134183). The collected species was registered in the Sistema Nacional de Gestão do Patrimônio Genético e Conhecimento Tradicional Associado (SisGen) under No. ACB5243.

Extraction and isolation procedures

The dried roots (1.621 kg) of *B. virgilioides* were powdered in a Thomas Wiley Laboratory Mill-Model4 mill, and sequentially, the material was submitted to maceration with MeOH by 48 h four times consecutively. The solvent was eliminated in a rota evaporator furnishing the MeOH extract (116.5 g). After dissolving this extract in a MeOH:H_2O (7:3) solution, the extract was partitioned between the hydroalcoholic solution and CH_2Cl_2, furnishing the DCM (10.8 g) and MeOH soluble fractions. Otherwise, the MeOH soluble fraction was diluted with H_2O and partitioned with EtOAc, obtaining the EtOAc soluble fraction (30.0 g).

The chromatographic column of the DCM phase (10.8 g) eluted with mixtures of $CHCl_3$:MeOH furnished 75 fractions of 50 mL each. Their CCDC permitted to join all of them in 12 sub-fractions due to the similarity after visualization in the UV lamps, iodine, and Lieberman-Buchard reagent. The fractions eluted with $CHCl_3$:MeOH (98:2) furnished 362 mg of a mixture of free fatty acids (FFA). The fractions eluted with $CHCl_3$:MeOH (96:4) were purified in a Sephadex LH-20 column eluted with DCM:MeOH furnishing **2** (7.0 mg) and **3** (90.0 mg). The following jointed fractions (110 mg), also eluted with the same eluent, were precipitated with MeOH and furnished lupeol (**4**) and lupenone (**5**). The fractions (1.207 g) eluted with $CHCl_3$:MeOH (95:5) were chromatographed in Si gel flash (hexane:EtOAc) and furnished 88.0 mg of a mixture of phytosterols β-sitosterol (**6**) and stigmasterol, besides sitostenone (**7**, 35.1 mg). This column also furnished a mixture of triglycerides (112.0 mg) that were identified by GC-MS analysis after derivatization reaction to obtain methyl fatty acids.

Transesterification procedure

The transesterification reaction was conducted on a fraction containing a mixture of triglycerides in a two-stage procedure. Initially, the reaction was assisted using a 0.5 M sodium methoxide solution with 2.0 mL of methanol and 30 mg of triglycerides, stirring for 30 min. Subsequently, H_2O was introduced to the mixture, and the methyl esters were extracted by partitioning between hexane, achieving an approximate yield of 78%. The final reaction product underwent analysis through gas chromatography flame ionization detection (GC-FID) and GC-MS to ascertain the specific composition of the methyl esters and any steroids present.

Antioxidant activity - 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Determining the antioxidant activity of MeOH, EtOAc, and DCM soluble fractions of the MeOH was carried out as previously reported with few adaptations.[15,16] A methanolic stock solution of DPPH (40 μg mL^{-1}, Sigma, Burlington, MA, USA) and solutions with the sample extracts (50, 150, 250, and 500 μg mL^{-1}) in methanol were prepared. The absorbance measurements of the reaction mixtures (300 μL of extract solution or standard or methanol for control and 2.7 mL DPPH stock solution) were performed at 515 nm, immediately after 30 min of incubation of the reaction at room temperature, protected from light, in a spectrophotometer (Cary 50 conc., Varian, Australia). Quercetin, Trolox, gallic acid, and butylated hydroxytoluene (BHT) were used as standards and were prepared in the same way as the extracts. All analyses were performed in triplicate.

The percentage of free radical scavenging (%FRS) was calculated as $\%FRS = [SA(CA - SA)/CA] \times 100$, where CA = final control absorbance, SA = final sample absorbance.

The efficient concentration, the amount of antioxidant needed to reduce the initial concentration of DPPH by 50% (EC_{50}), was calculated by linear regression, where the abscissa axis represented sample (μg mL^{-1}) or positive control concentrations and ordinate the percentage of free radical sequestration (%FRS). When necessary, dilutions were carried out at the concentrations of the samples and the standard to enable the calculation of the EC_{50}.

Antioxidant assay using the β-carotene/linolenic acid method

The evaluation of the antioxidant activity using the β-carotene/linolenic acid system was performed according to a previously described method.[16] A 2 mg of β-carotene solution in 1 mL of $CHCl_3$ was pipetted into a flask containing 20 mg of linolenic acid and 200 mg of Tween 80. The solvent was removed from the mixture by rotary evaporation, and then 200 mL of distilled water was added, stirring to promote aeration and forming an emulsion. A 2.7 mL aliquot of the emulsion was added to a cuvette containing 300 mL of the extracts or standards (100, 150, 200, and 250 μg mL^{-1} in methanol), and the absorbance was measured at 470 nm with immediate reading and 15-min intervals during 1 h. The cuvettes were incubated in a water bath at 50 °C. The control was prepared with 2.7 mL of the oxidizing medium and 300 mL of methanol. All analyses were performed in triplicate, using quercetin as standard, at the same concentrations of the extracts. Antioxidant activity (AA) was calculated using the following equation: $AA = (DR_C - DR_S)/DR_C \times 100$, where DR_C is the degradation rate of the control ($DR_C = \ln(a/b)/60$), DR_S is the degradation rate in the presence of the sample ($DR_S = \ln(a/b)/60$), a is the initial absorbance at time 0, and b is the absorbance at 60 or 30 min. The EC_{50} was calculated by linear regression, where the abscissa axis represented sample (μg mL^{-1}) or positive control concentrations and ordinate the percentage of antioxidant activity (%AA). When necessary, dilutions were carried out at the concentrations of the samples and the standard to enable the calculation of the EC_{50}.

Brine shrimp test

For an initial bioactivity screening, the adapted brine shrimp lethality assay described with minor modifications[17] was employed to study the general cytotoxicity of compounds **1**-**6**. Alive *Artemia salina* Leach cysts (500 mg) were transferred to a conical flask containing 3500 mL of artificial seawater. The flasks were aerated with an air aquarium pump and kept at 25 °C under a 60 W incandescent lamp. After 48 h, the nauplii hatched and then the MeOH extract

was dissolved in artificial seawater with DMSO (5%) to reach the final concentration of 1 mg mL^{-1}. Solutions of 50, 100, 150, 200, and 250 µg mL^{-1} of extract (25, 50, 75, 100, and 150 µg mL^{-1}) were transferred to vials containing seawater (5 mL) and 10 nauplii. DMSO, seawater, and 10 nauplii were the negative controls. The assays were carried out in triplicate. After 24 h of incubation, the number of live nauplii was counted. The mortality was defined as the absence of controlled forward motion during 30 s of observation. The lethal concentration doses for 50% of the brine shrimp (LC_{50}) and the respective 95% confidence intervals were determined using the GraphPad Prism version 5.0 software.[18]

*Cordoin (**1**)*

HRESIMS, $[M + H]^-$ *m/z* 323.1281; IR (KBr) ν cm^{-1} 3448, 1743, 879; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.45 (1H, s, OH), 7.90 (1H, d, *J* 15.0 Hz, H-β), 7.72 (1H, d, *J* 9.0 Hz, H-6'), 7.64 (2H, m, H-2 and H-6), 7.60 (1H, d, *J* 15.0 Hz, H-α), 7.41 (2H, m, H-3 and H-5), 6.51 (1H, d, *J* 9.0 Hz, H-5'), 5.47 (1H, t, *J* 7.0 Hz, H-2"), 4.47 (1H, d, *J* 7.0 Hz, H-1"), 1.83 (3H, s, H-4"), 1.80 (3H, s, H-5"); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 192.41 (C-β'), 166.73 (C-2'), 165.76 (C-4'), 144.53 (C-β), 136.00 (C-3"), 134.98 (C-1), 131.28 (C-4 and C-6'), 129.78 (C-2 and C-6), 128.81 (C-3 and C-5), 121.45 (C-2"), 120.80 (C-α), 114.05 (C-1'), 108.83 (C-5'), 101.79 (C-3'), 26.11 (C-5"), 21.86 (C-1"), 18.45 (C-4").

*Isocordoin (**2**)*

IR (KBr) ν cm^{-1} 3151, 1631, 856, 750, 700; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 13.75 (1H, s, OH), 7.88 (1H, d, *J* 15.0 Hz, H-β), 7.74 (1H, d, *J* 9.0 Hz, H-6'), 7.65 (2H, m, H-2 and H-6), 7.60 (1H, d, *J* 15.0 Hz, H-α), 7.43 (2H, m, H-3 and H-5), 6.44 (1H, d, *J* 9.0 Hz, H-5'), 5.31 (1H, t, *J* 7.0 Hz, H-2"), 3.49 (1H, d, *J* 7.0 Hz, H-1"), 1.85 (3H, s, H-4"), 1.78 (3H, s, H-5"); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 192.11 (C-β'), 163.87 (C-2'), 161.43 (C-4'), 144.12 (C-β), 135.43 (C-3"), 134.73 (C-1), 130.33 (C-6'), 128.86 (C-2 and C-3), 128.26 (C-4 and C-6), 121.05 (C-5), 120.00 (C-α and C-2"), 114.22 (C-3'), 113.75 (C-1'), 107.87 (C-5'), 25.70 (C-5"), 21.64 (C-1"), 17.75 (C-4").

*Lupeol (**3**)*

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 151.52 (C-20), 109.88 (C-29), 79.41 (C-3), 55.22 (C-5), 50.80 (C-9), 48.72 (C-18), 48.33 (C-19), 43.20 (C-17), 41.20 (C-14), 40.50 (C-8 and C-22), 39.20 (C-4), 39.13 (C-13), 38.11 (C-1), 37.71 (C-10), 35.80 (C-16), 34.72 (C-7), 28.40 (C-21), 27.90 (C-23 and C-15), 27.43 (C-2), 25.07 (C-12), 21.42 (C-11), 19.62 (C-30), 18.35 (C-6), 18.00 (C-28), 16.50 (C-26), 16.42 (C-25), 15.80 (C-24), 14.90 (C-27).

*Lupenone (**4**)*

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 218.20 (C-3), 151.00 (C-20), 109.35 (C-29), 54.80 (C-5), 49.70 (C-9), 48.20 (C-18), 47.92 (C-19), 47.30 (C-4), 42.80 (C-14), 40.70 (C-8), 39.90 (C-22), 39.50 (C-1), 38.10 (C-13), 36.90 (C-10), 35.50 (C-16), 34.10 (C-2), 33.50 (C-7 and C-16), 29.71 (C-21), 27.40 (C-15), 26.60 (C-23), 25.11 (C-12), 21.40 (C-11), 21.00 (C-24), 19.70 (C-6), 19.30 (C-30), 17.90 (C-28), 15.90 (C-26), 15.70 (C-25), 14.40 (C-27).

*Sitostenone (**7**)*

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 199.69 (C-3), 172.11 (C-5), 123.48 (C-4), 55.85 (C-14 and C-17), 53.78 (C-9), 45.85 (C-24), 42.25 (C-13), 39.75 (C-10), 38.60 (C-12), 36.13 (C-1 and C-20), 35.60 (C-8), 35.59 (C-22), 33.91 (C-2 and C-6), 32.95 (C-7), 29.60 (C-25), 28.24 (C-16), 26.10 (C-23), 24.94 (C-15), 23.03 (C-28), 21.00 (C-11), 20.90 (C-26), 19.70 (C-27), 18.69 (C-19 and C-21), 11.85 (C-18), 11.01 (C-29).

RESULTS AND DISCUSSION

Analysis of the NMR and IR data of the first eluted fraction of the root extract of *B. virgilioides* indicated that it is composed of a mixture of free fatty acids. This conclusion was based on the absence of peaks of the oximethyne and oximethylenes hydrogens and carbons in the ^{1}H and ^{13}C NMR spectra of this mixture (Figure 1). Besides, the ^{13}C NMR showed peaks of carboxylic acyl groups and olefinic carbons at δ 181.0 and 127-131 ppm, characteristics peaks of carboxylic acids and unsaturated chains (Figure 1). The other peaks of the spectra and the integrals revel this mixture is composed mainly by linear saturated and unsaturated fatty acids, including very long fatty acids (VLFA). The ESIMS registered in positive and negative modes permitted tentatively identifying the composition, considering the biosynthetic pathway with the double bond in C-9. Comparing the molecular ions registered with linear chains of fatty acid, it was possible to propose the presence of C:16 to C:42 chains (Table 1). Fatty acids are

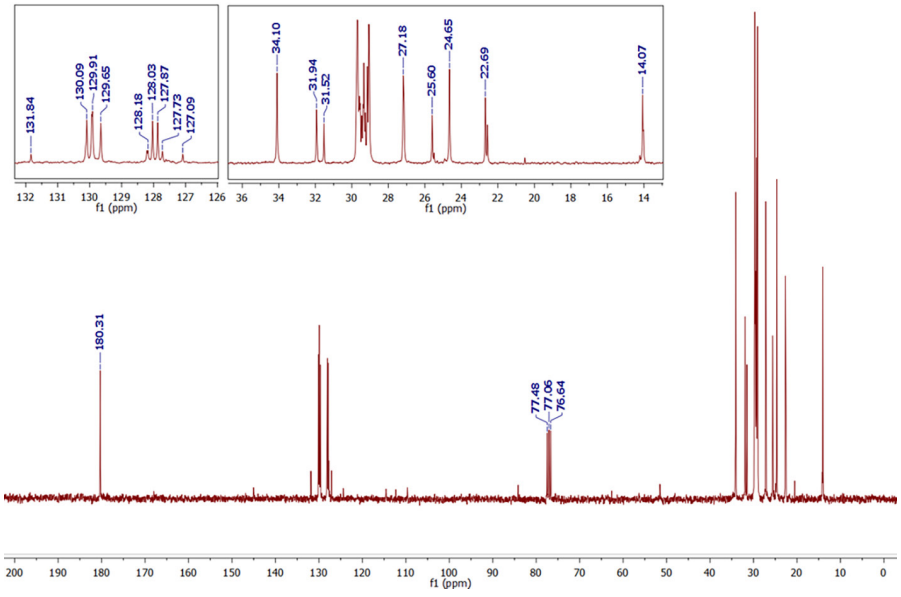

Figure 1. *^{13}C NMR spectra of a mixture of FFA [125 MHz, $CDCl_3$, δ (ppm)]*

ubiquitous, and as such, they belong to a physiologically important class of molecules involved in cell energy storage (e.g., adipose tissues), membrane structure, and various signaling pathways. VLFA are the fatty acids with more than C:18. Besides being involved as membrane constituents and signaling molecules in sphingolipids and phospholipids, they are also required in cuticular waxes and suberin formations, two extracellular biopolymers preventing plants from desiccation or external aggressions.[19] However, the presence of free fatty acids is not regular in plants, and few studies deal with the presence of FFA and VLFA in plants. As examples, these classes of compounds were detected previously in sugar cane wax[20] and in different peel variety grapes,[21] and the authors infer they are produced by habitat stress, suggesting the FFAs are oxidative substrates by mitochondria and participate in various electron transfer pathways. It may be one of the adaptation mechanisms.[22] Another role for these compounds is that plants have evolved strategies to prevent water loss under drought stress, and the increment of wax accumulation in leaves could enhance drought resistance.[23]

In order to compare the composition of the FFA with the esterified fatty acid in the triglyceride fraction, after transesterification of the glycerides, the methyl fatty esters were analyzed by GC-MS and compared with a FAME standard mixture. The results indicated that the primary fatty acids present in the triglyceride fractions were decanoic, heptadecanoic, heptadecenoic, octadecanoic (stearic), octadecenoic (oleic), and eicosanoic acids, confirming the uncommon nature of the FFA mixture.

Table 1. Free fatty acids from *B. virgilioides* roots annotated from positive and negative ESI mass spectrometry

Fatty acid	Negative *m/z* Cluster $[M - H]^-$	Positive *m/z*	Cluster
C16:0	254.77		
C16:1		293.26	$[M + Na]^+$
C17:0	268.82		
C18:1	281.0	305.26/ 321.28	$[M + Na]^+/[M + K]^+$
C18:2	278.83	303.26/319.27	$[M + Na]^+/[M + K]^+$
C18:3	277.0	301.24/317.27	$[M + Na]^+/[M + K]^+$
C19:0	296.85	337.28	$[M + K]^+$
C19:1	294.85		
C19:2	292.81		
C20:0	310.9	335.27/351.26	$[M + Na]^+/[M + K]^+$
C20:1		333.27/349.26	$[M + Na]^+/ [M + K]^+$
C21:0	324.93		
C22:0	339.03		
C22:1		361.19	$[M + K]^+$
C24:0	367.14		
C24:1		327.26	$[M + H]^+$
C30:0		491.32	$[M + K]^+$
C32:1		479.37	$[M + H]^+$
C34:1		507.39/545.39	$[M + H]^+/[M + K]^+$
C35:1		521.40	$[M + H]^+$
C36:0	535.43	575.4	$[M + K]^+$
C36:1	533.33	573.6	$[M + K]^+$
C36:2		561.4	$[M + K]^+$
C37:0	549[a]		
C38:0	563.45		
C38:1	561.47		
C38:2	559.48	599.52	$[M + K]^+$
C38:3	557.45		
C37:1		577.41	$[M + H]^+$
C39:0	577.48	601.3/617.5	$[M + Na]^+/[M + K]^+$
C39:2	573.45		
C42:1		647.51	$[M + K]^+$

[a]Approximated values.

On the other hand, the chromatographic procedures permitted to isolate flavonoids, steroids, and triterpenes (Figure 2) identified as cordoin (**1**), isocordoin (**2**), lupeol (**3**), lupenone (**4**), β-sitosterol, stigmasterol, and sitostenone (**7**). All of them were identified by NMR mono and bidimensional, infrared, and mass spectrometry data analysis.

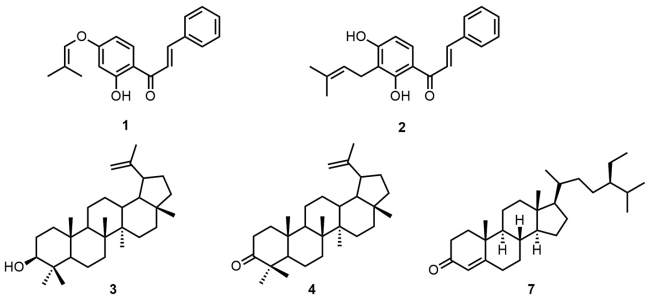

Figure 2. *Chalcones, triterpenes, and steroids isolated from B. virgilioides roots.* ***1****: Cordoin;* ***2****: isocordoin;* ***3****: lupeol;* ***4****: lupenone;* ***7****: stigmasterol*

Compounds **1** and **2** were obtained in the mixture and the spectrometric data permitted to identify them without purification. Their negative ESI HRMS showed just one molecular ion at *m/z* 307.1343 ($C_{20}H_{20}O_3$, requires 307.1334). The NMR analysis of the two compounds was carried out by ^1H, ^{13}C, HSQC (heteronuclear single quantum correlation), and HMBC (heteronuclear multiple bond correlation) experiments that permitted identifying each structure's hydrogen and carbons. The correlations observed in the counter map of bidimensional spectra of oxymethylene hydrogens (H-2") and the hydrogenated carbons C-5' and C-3' for compound **1** and methylene hydrogens (H-2") and the oxygenated aromatic carbons C-5' and C-3' of **2** permitted to discriminate each one. Besides, comparison of the data with literature[24,25] permitted identify unequivocally these chalcones named cordoin (**1**) and isocordoin (**2**). The peak intensities of NMR spectra indicated cordoin is the majority compound in the mixture. Isocordoin has already been isolated in Fabaceae spp.; however, cordoin was previously isolated in *Lonchocarpus* spp. (*Cordoa* spp.), and this study is the first record of the occurrence of isocordoin and cordoin in *Bowdichia*.

Lupeol (**3**), lupenone (**4**), and sitostenone (**7**) were directly identified by comparison of NMR registered data with literature data for these compounds.[26,27] The mixture of sitosterol and stigmasterol was identified by TLC comparing with pure standards and also NMR data analysis.

The extracts were submitted to antioxidant evaluation (quenching DPPH and co-oxidation of β-carotene/linolenic acid system), and the results showed a moderate antioxidant capacity (Figures 3 and 4). The hexane soluble fraction showed a lower antioxidant value, and this result indicates the VLFA and triglycerides are not active. The MeOH and EtOAc were the best extracts due to the presence of phenolics in their composition.

Concerning the toxicity of extract *Artemia salina* nauplii, the results showed that the MeOH soluble fraction was considered very toxic with a LC_{50} (lethal concentration) 76.82 μg mL^{-1}.[28] This test is a screen for cytotoxic and insecticidal evaluation of metabolites, and it is helpful due to its simplicity, low cost, and correspondence with the *in vitro* activity in cells. Cordoin is known to inhibit 90% of CDC25B in oncogenic enzymes, and its overexpression has been described in several types of human cancers.[29] Analysis of the effects of cordoin on two human colon cancer cell lines (HTC-116 and DLD-1) demonstrated that treatment with this compound (30 μmol L^{-1})

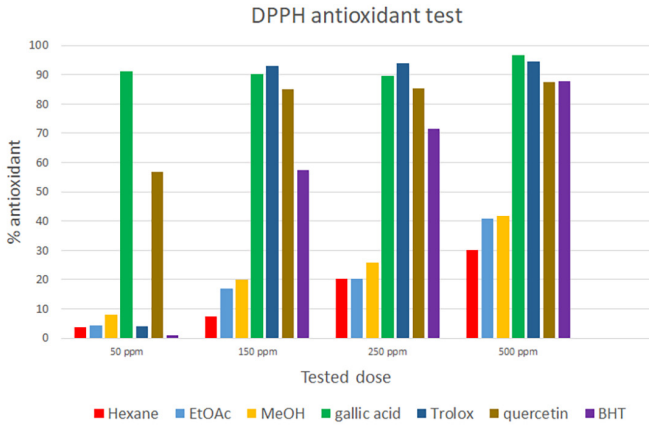

Figure 3. *DPPH quenching test for the organic extracts of B. virgilioides roots*

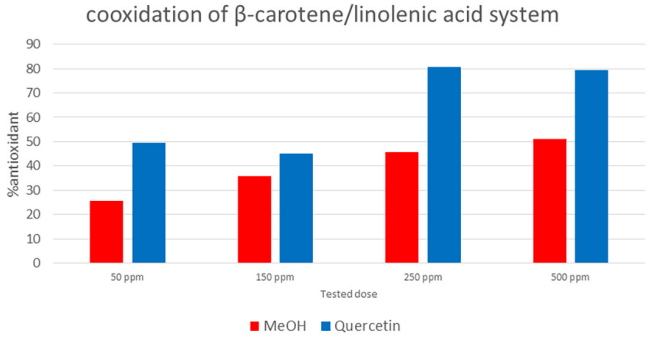

Figure 4. *Autooxidation of β-carotene inhibition for the organic extracts of B. virgilioides roots*

reduced by 40 and 50% the cell proliferation of HTC-116 and DLD-1 strains, respectively, after 24 h of exposure.[30]

CONCLUSIONS

This paper reports the chemical composition of *B. virgilioides*, a plant belonging to the Fabaceae family. From the organic extracts of roots, unusual very long free fatty acids were obtained and characterized. These compounds were mainly absent from the fatty acid triglycerides. Moreover, two prenyl chalcones (cordoin and isocordoin) were isolated from the *Bowdichia* genus for the first time. The extracts showed low antioxidant activities, but the MeOH extract exhibited remarkable cytotoxicity in the brine shrimp assay.

SUPPLEMENTARY MATERIAL

Complementary material for this work is available at http://quimicanova.sbq.org.br/, as a PDF file, with free access.

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