http://dx.doi.org/10.21577/0103-5053.20190076

J. Braz. Chem. Soc., Vol. 30, No. 8, 1728-1740, 2019
Printed in Brazil - ©2019 Sociedade Brasileira de Química

Article

Chemical Constituents, Antiproliferative and Antioxidant Activities of *Vernonanthura nudiflora* (Less.) H. Rob. Aerial Parts

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Sesquiterpene lactones are an important class of secondary metabolites frequently isolated from *Vernonanthura* genus that present a variety of biological properties, including antiproliferative activity. Due to the limitation of pharmacological studies on *Vernonanthura nudiflora*, the aim of this work was to investigate their antioxidant potential and antiproliferative activity against human tumor cells, as well as to isolate and identify the chemical constituents present in their aerial parts. The phytochemical investigation resulted in the isolation of the sesquiterpene lactones piptocarphins A, B, D, and a new hirsutinolide derivative, 8α-tigloyloxy-10α-hydroxy-hirsutinolide, besides triterpenes, glycosylated steroids, flavonoids, and one chlorogenic acid derivative. Also, other sesquiterpene lactones were identified by ultra-high performance liquid chromatography-high resolution tandem mass spectrometry (UHPLC-HRMS/MS) from dichloromethane fraction. This fraction showed activity against the tumor cells tested, mainly against leukemia, glioma, ovarian and kidney, with growth inhibitory activity (GI_{50}) less than 0.80 μg mL^{-1}. Piptocarphins A and B, in mixture, showed strong activity against all human cancer cell lines tested, with GI_{50} values ≤ 0.15 μg mL^{-1}. Piptocarphin D was selective for glioma and resistant ovarian cell lines. The new hirsutinolide derivative showed potent activity against breast (GI_{50} = 0.96 μg mL^{-1}) and resistant ovarian (GI_{50} = 3.60 μg mL^{-1}) cell lines.

Keywords: Asteraceae, *Vernonanthura nudiflora*, sesquiterpene lactones, new hirsutinolide, pharmacological potential

Introduction

Vernonanthura H. Rob. (Asteraceae family) comprises approximately 70 species widely distributed from southern Mexico to central Argentina,[1-4] of which 41 occur in Brazil, concentrated mainly in South and Southeast regions.[5]

Some species now assigned to *Vernonanthura* were originally placed in *Vernonia* genus. Robinson[4] segregated most of the South America species in 22 new genera, including *Vernonanthura*. Several *Vernonanthura* species are known in Brazil as "assa-peixe", and are used in traditional medicine for the treatment of flu and colds.[6,7] Species of this genus have also been reported to possess pharmacological activities,[8] such as antiplasmodial,[9] antileishmanial,[10-12] antimicrobial,[13-15] antioxidant,[11] antinociceptive and anti-inflammatory.[16,17]

Vernonanthura nudiflora (Less.) H. Rob., popularly known as "alecrim do campo", is a sub-shrub, 50-80 cm

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high, flowering showy, native to South America, with distribution in Argentina, Brazil and Uruguay.[5,18,19] A provoked experimental intoxication in sheep showed that *V. nudiflora* has moderate toxic action on the digestive system of this animal, nevertheless no significant effects on the respiratory and circulatory system were observed.[20] Previous experiment undertaken by Dobereiner and Tokarnia[21] also showed that *V. nudiflora* provoke irritation on the digestive tract mucosa of cattle and sheep.

V. nudiflora ethanolic extract presented potent antiproliferative activity against leukemia tumor cell lines, evidencing promising therapeutic potential.[22] Previous phytochemical investigation of this species revealed the presence of triterpenes, steroids, flavonoids, and mainly sesquiterpene lactones of glaucolide, hirsutinolide and cadinanolide classes.[23,24] Sesquiterpene lactones are still an important class of secondary metabolites, providing new therapeutic leads, especially for development of anti-inflammatory and anticancer agents.[8,25,26]

Hirsutinolide-type sesquiterpene lactones isolated from natural products, and semi-synthetic analogues, showed *in vitro* and *in vivo* activity against human glioma cell lines.[27] Additionally, Youn *et al.*[28] reported that hirsutinolides isolated from *Vernonia cinerea* were able to inhibit glioblastoma (U251MG), and breast tumor cells (MDA-MB-231), which demonstrate the potential of this class of compounds.

In this paper, we describe the isolation and structural elucidation of one new hirsutinolide derivative, along with sixteen known compounds. In addition, other nine sesquiterpene lactones were identified by ultra-high pressure liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS/MS) from dichloromethane fraction. Moreover, due to the limitation of pharmacological studies on *V. nudiflora*, the evaluation of their antioxidant potential, and antiproliferative activity against human tumor cells, were carried out.

Experimental

General experimental procedures

Chromatography separations were performed on silica gel 60 (70-230 mesh, Merck), silica gel flash (230-400 mesh, Acros Organics), Sephadex LH-20® (Sigma) or polyvinylpolypyrrolidone (PVPP, Sigma-Aldrich) chromatography columns (CC). Thin layer chromatography (TLC) was performed on normal phase pre-coated silica gel 60G or $60GF_{254}$ (Merck) plates. Visualization of the compounds on TLC was accomplished by UV irradiation at 254 and 366 nm, and/or by spraying with H_2SO_4/anisaldehyde/acetic acid (1:0.5:50 mL) solution followed by heating at 100 °C. HPLC separations were performed on a Shimadzu instrument (Mod. Prominence) with two LC-20AR pumps, degasser DGU-20ASR, detector UV-Vis SPD-M20A model and injection system automatic SIL-10AF, and equipped with a Shim-pack PREP-ODS (250 × 20 mm; 15 μm) column. The mobile phase consisted of water (Milli-Q, Millipore) and methanol (Sigma Chemicals Co). Nuclear magnetic resonance (NMR) spectra were recorded on a VARIAN Mercury Plus spectrometer operating at 300 and 75.5 MHz, and Bruker avance III HD spectrometer operating at 500 and 125 MHz, using $CDCl_3$ and dimethyl sulfoxide (DMSO-d_6) as solvents. The UHPLC analysis was performed in a Shimadzu Nexera X2 instrument, equipped with a CBM-20A a system controller, two LC-30AD pumps, a CTO-30A column oven and SIL-30AC autosampler. The mass spectra were recorded on Bruker IMPACT II mass spectrometer, with electrospray ionization source (ESI) in the positive ion mode, quadrupole-time of flight (Q-TOF) analyzer and multichannel plate (MCP) detector. The optical rotation was measured on a PerkinElmer polarimeter at 24 °C and λ = 589 nm (sodium D-line), using a 1 cm microcell (*c* 0.15, $CHCl_3$). UV-Vis spectra were recorded using a PHARO 300 spectrophotometer (Merck). The solvent was removed using Rocket Synergy sample concentrator (Genevac).

Plant material

The aerial parts of *V. nudiflora* were collected at Ponta Grossa city, Paraná State, Brazil (25°05'16" S, 50°05'43" W) on March 2016, and indentified by Dr Marta Regina Barrotto do Carmo. A voucher specimen was deposited at the herbarium at Universidade Estadual de Ponta Grossa (HUPG 21694).

Extraction and isolation

Air-dried aerial parts of *V. nudiflora* (765.0 g) were ground, exhaustively extracted with ethanol at room temperature and concentrated under reduced pressure at 37 ºC, to yield the crude extract (CE, 42.0 g). A part of the crude extract (38.0 g) was suspended in MeOH/H_2O (1:1, v/v, 400 mL), and successively partitioned with *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol. The solvents were removed under reduced pressure to give *n*-hexane (HE; 16.0 g), dichloromethane (DC; 3.7 g), ethyl acetate (EA; 4.2 g), butanolic (BU; 6.6 g) and residual hydromethanolic (HM; 6.8 g) fractions.

An aliquot of HE fraction (7.0 g) was submitted to vacuum liquid chromatography (VLC) (ϕ (internal diameter) = 4.9 cm × h (heigth) = 14.8 cm on silica gel

column, using a gradient of hexane/EtOAc, to afford the subfractions HE-1 to HE-13. The subfraction HE-2 (1.65 g) was subjected to silica gel CC (ϕ = 3.0 cm × h = 26.1 cm) using *n*-hexane/EtOAc gradient as eluents, in order to increasing gradient of polarity, and afforded compound **1** (4.2 mg). Subfraction HE-3 (0.5 g) afforded a mixture of compounds **1-5** (25.2 mg) after CC (ϕ = 2.3 cm × h = 27.0 cm) in silica gel flash using *n*-hexane/EtOAc gradient as eluent. Subfraction HE-5 (150.0 mg) was also submitted to silica gel CC (ϕ = 1.8 cm × h = 22.8 cm) using *n*-hexane/EtOAc gradient as eluent, resulting in twelve fractions (HE-5-1 to HE-5-12). Subfraction HE-5-8 (50.0 mg) was fractionated by CC on silica flash (ϕ = 1.1 cm × h = 27.1 cm) with *n*-hexane/EtOAc gradient, affording compounds **6** and **7** (1.0 mg) in mixture.

An aliquot of DC fraction (1.20 g) was submitted to silica gel CC (ϕ = 2.6 cm × h = 29.6 cm) eluted with *n*-hexane/$CHCl_3$/MeOH gradient giving subfractions DC-1 to DC-15. Subfraction DC-5 afforded compound **10** (3.5 mg). Subfraction DC-6 (370.4 mg) was subjected to silica flash CC (ϕ = 1.9 cm × h = 23.5 cm) using *n*-hexane/acetone gradient as eluents to afford the subfractions DC-6-1 to DC-6-11. Subfractions DC-6-3 (41.4 mg) and DC-6-4 (75.9 mg) were submitted to purification on Sephadex LH-20 CC (ϕ = 0.8 cm × h = 18.6 cm) using $CHCl_3$/MeOH (2:8, v/v) isocratic as mobile phase. Compounds **6** and **7** (10.5 mg, in mixture) and compounds **11** and **12** (2.5 mg, in mixture) were identified in subfractions DC-6-4-3 and DC-6-4-6, respectively. Subfractions DC-6-3-2 (51.8 mg) were submitted to semi-preparative HPLC purification (conditions: Shim-pack PREP-ODS (250 × 20 mm; 15 μm) column, mobile phase MeOH:H_2O (1:1, v/v), 15 mL min^{-1} flow, analysis time: 30 min and λ = 254 nm) affording compounds **8** (2.7 mg, t_R = 10 min) and compound **9** (2.4 mg, t_R = 11 min), and the reisolation of compounds **6** (2.7 mg, t_R = 12 min) and **7** (1.4 mg, t_R = 15 min). Subfraction DC-9 (56.3 mg) was purified by recrystallization in acetone to yield compounds **13** and **14** (9.0 mg) in mixture.

An aliquot of EA fraction (2.0 g) was submitted to Sephadex LH-20® CC (ϕ = 2.1 cm × h = 23.2 cm) using MeOH/H_2O, in decreasing polarity gradient, to obtain subfractions EA-1 to EA-16. Further purification of EA-3 (180.6 mg) using Sephadex LH-20® CC (ϕ = 1.1 cm × h = 24.1 cm) and MeOH/H_2O (1:1, v/v) isocratic as mobile phase, resulted in the isolation of compound **15** (14.6 mg). Compound **16** (35.6 mg) was isolated from subfraction EA-12 (215.6 mg) after CC in Sephadex LH-20® (ϕ = 2.3 cm × h = 23.0 cm) using MeOH/H_2O in decreasing polarity gradient. Compounds **11** and **12** (6.5 mg, in mixture) and **16** (22.4 mg) were reisolated from EA-14 fraction (48.6 mg) after TLC preparative purification using $CHCl_3$:MeOH (6:4, v/v) as mobile phase. Subfraction EA-15 (52.2 mg) was submitted to a PVPP CC (ϕ = 1.2 cm × h = 7.5 cm), using MeOH (isocratic elution), to yield compound **17** (3.0 mg). Compound **15** (6.8 mg) and compound **16** (21.6 mg) were reisolated from HM fraction (1.50 g) after CC in Sephadex LH-20® CC (ϕ = 2.1 cm × h = 23.2 cm) using MeOH/H_2O in decreasing polarity gradient.

Analysis of the dichloromethane fraction by UHPLC-HRMS/MS

The sample was prepared in MeOH (1.0 mg mL^{-1}) and chromatographic separations were performed using UHPLC on a Symmetry C18 column (75 × 2.0 mm i.d.; 1.6 μm Shim-pack XR-ODS III), maintained at a temperature of 40 ºC. The mobile phase consisted of H_2O (solvent A) and 0.1% formic acid in CH_3CN (solvent B). The gradient program was as follows: initial 0-1 min, using elution A-B (95:5, v/v), 1-3 min (30:70 v/v), 3-12 min (5:95 v/v) and kept at 95% B for 16 min at a flow rate of 0.2 mL min^{-1}. Injection volume was 3 μL. High resolution mass spectrometry analysis were carried out in a Q-TOF mass spectrometer via an electrospray ionization interface. The capillary voltage was operated in positive ionization mode, set at 4500 V, using sodium formate (10 μM) as calibrant. The dry gas parameters were set to 8 L min^{-1} at 200 ºC with a nebulization gas presure of 4 bar. Colision-induced dissociation (CID) fragmentation was performed using argon (Ar) collision gas and collision energy from 0-30 eV. Spectra data of the investigated compounds were collected from *m/z* 50-1300 with a resolution of 50.000, and with an acquisition rate of 5 spectrums *per* second. The ions of interest were selected by auto MS/MS scan fragmentation. The data processing software was Data analysis 4.3 (Bruker). Moreover, the mass error value was calculated. Only molecular formulas ≤ 5 ppm of error were considered in this study.[29]

In vitro antiproliferative assay

In vitro antiproliferative activity experiments on human cell lines were performed according to Monks *et al.*[30] The crude extract and fractions of *V. nudiflora* were evaluated *in vitro* against ten human tumor cell lines [U251 (glioma, CNS), UACC-22 (melanoma), MCF-7 (breast), NCI-ADR/RES (ovarian expressing the multiple drug resistance phenotype), 786-0 (renal), NCI-H460 (lung, non-small cells), PC-3 (prostate), OVCAR-3 (ovarian), HT-29 (colon), and K-562 (leukemia)], kindly provided by the National Cancer Institute (Frederick, MA, USA). More, some of the isolated compounds (**6** + **7**, **8**, **9**, **10**) were tested

against six human tumor cell lines [U251 (glioma, CNS), MCF-7 (breast), NCI-ADR/RES (ovarian expressing the multiple drug resistance phenotype), NCI-H460 (lung, non-small cells), PC-3 (prostate), HT-29 (colon)]. All samples were also evaluated against the immortalized human keratinocytes (HaCat) cell line (provided by Prof Dr Ricardo Della Coletta, UNICAMP). Stock solution (0.1g mL^{-1}) of each samples was prepared in DMSO and successively diluted in RPMI 1640, supplemented with 5% fetal bovine serum and 1% penicillin:streptomycin mixture (1000 UI mL^{-1}:1000 μg mL^{-1}) to final concentrations (0.25, 2.5, 25 and 250 μg mL^{-1} for the extract and fractions, and 0.15, 1.50, 15.0 and 150.0 μg mL^{-1} for the isolated compounds). The chemotherapeutic doxorubicin chloridrate (0.025, 0.25, 2.5 and 25 μg mL^{-1}) was used as a positive reference standard to determine the sensitivity of cell lines. Cells in 96-well plates (100 μL cells *per* well) were exposed to sample concentrations, in triplicate, for 48 h at 37 °C and 5% of CO_2. The final DMSO concentration (≤ 0.25%) did not affect cell viability. Before (plate control) and after sample addition, cells were fixed with 50% trichloroacetic acid, and cell proliferation was determined by spectrophotometric quantification of cellular protein content at 540 nm (Molecular Devices, model VersaMax) using sulforhodamine B. Two effective concentrations, named growth inhibition 50% (GI_{50}) and total growth inhibition (TGI), were calculated by non-linear regression analysis (sigmoidal fit) using Origin 7.5® (OriginLab Corporation).[31] The selectivity index (SI) was calculated as SI = GI_{50} HaCat / GI_{50} tumor cell line.[32]

In vitro antioxidant assays

DPPH free-radical scavenging activity

The free radical scavenging effect of the crude extract and fractions of *V. nudiflora* was investigated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) (Sigma-Aldrich) assay, based on the method proposed by Boroski *et al.*[33] with slight modifications. Serial concentrations (5, 10, 25, 50, 75, 100, 150 and 250 μg mL^{-1}) of extract and fractions were prepared by addition of 20 mg of samples and 10 mL of methanol and 2 mL of DPPH methanolic solution (3.20 mg DPPH in 100 mL) were mixed with these solutions. The mixture was thoroughly vortex-mixed and kept in the dark for 30 min. Absorbance was measured using a UV-Vis spectrophotometer at 517 nm. Rutin and ascorbic acid were used as standards antioxidant compounds. All samples were tested in triplicate. The results were expressed as percent inhibition of the DPPH radical, which was calculated using the equation 1:

$$\% \text{ inhibition} = [(A_{DPPH} - A_{sample}) / A_{DPPH}] \times 100] \quad (1)$$

where A_{DPPH} is the absorbance of the DPPH solution and A_{sample} is the absorbance of the DPPH solution with the sample tested. The sample concentration that afforded 50% inhibition (IC_{50}) was obtained by plotting the concentrations of the sample solutions *versus* percent inhibition.

$ABTS^{\bullet+}$ radical scavenging activity

The ABTS method was performed according to method described by Re *et al.*[34] with some modifications. Briefly, 7.0 mM of ABTS (2,2-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid) (Sigma-Aldrich) and potassium persulfate (140 mM) were mixed, and keeping in the dark for 16 h at room temperature. For the analysis, the $ABTS^{\bullet+}$ solution was diluted in ethanol (PA) to afford the $ABTS^{\bullet+}$ work solution (absorbance of 0.700 ± 0.050 at 734 nm). Aliquots (30 μL) of each extract and fractions, at different concentrations in triplicate, were homogenized with $ABTS^{\bullet+}$ work solution (3 mL) and, after 6 min, the absorbance was measured at 734 nm using a UV-Vis spectrophotometer. The results were expressed as percent inhibition of the ABTS radical, using the equation described in the DPPH assay. Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid; Sigma Aldrich) was used as antioxidant standard.

Total phenolic compounds (TPC)

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method as described by Singleton and Rossi,[35] and adapted by Boroski *et al.*[36] One aliquot (0.25 mL) of each sample in MeOH (2.5 mg mL^{-1}) was diluted in distilled water (4 mL), and mixed with Folin-Ciocalteu reagent (0.25 mL) and saturated sodium carbonate solution (0.50 mL). This mixture was kept in the dark at room temperature (23-25 ºC) for 1 h before the absorption measurement at 765 nm. Gallic acid was used as standard in the calibration curve. Results were expressed as milligrams of gallic acid equivalents *per* gram of sample (mg GAE g^{-1} extract).

Total flavonoids (TF)

The total flavonoid (TF) content of crude extract and fractions was determined by aluminium chloride colorimetric method,[37] adapted by Boroski *et al.*[36] Aliquot (0.5 mL, in triplicate) of each sample diluted in MeOH (2.5 mg mL^{-1}) was mixed with 5% $AlCl_3$ solution (0.25 mL) and methanol (4.25 mL) in graduated centrifuge tubes (15 mL). The resulting mixture was allowed to rest for 30 min at room temperature, protected from light. After that, the absorbance at 415 nm was measured. Quercetin was used as standard.

The flavonoid content was expressed in mg of quercetin equivalent *per* gram extract (mg QE g^{-1} extract).

Statistical analysis

The results were submitted to variance analysis (ANOVA) and Tukey's test (5% probability) using the software Assistat® (version 7.7).[38]

Results and Discussion

Chemical investigation of *V. nudiflora* aerial parts resulted in the isolation of seventeen compounds (Figure 1). The structures of the compounds were assigned on the basis of NMR (^{1}H and ^{13}C NMR, heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC), nuclear Overhauser effect spectroscopy (NOESY)) and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS/MS) data, and comparison with data reported in the literature. Compounds **1** to **5** were identified as the pentacyclic triterpenes, lupeol (**1**), α-amyrin (**2**), β-amyrin (**3**), taraxasterol (**4**) and pseudotaraxasterol (**5**).[39] Compounds **6** to **8** were identified as the known hirsutinolide sesquiterpene lactones, piptocarphins A (**6**), B (**7**), D (**8**)[40] (Table 1), while compound **9** was identified as the new hirsutinolide derivative, 8α-tigloyloxy-10α hydroxy-hirsutinolide (Table 1). The configuration for these hirsutinolides was established by comparing the chemical shifts and the coupling constants.[41,42] Compounds **13** and **14** were obtained as mixture and characterized as the glycosylated steroids, 3-*O*-β-*D*-glucopyranosyl-β-sitosterol and 3-*O*-β-*D*-glucopyranosyl-stigmasterol, respectively.[43] Compounds **10**, **11**, **12**, **16** and **17** were identified as the flavonoids velutin,[44] apigenin, chrysoeriol,[45,46] rutin[45,47] and luteolin,[48] respectively. Compound **15** were identified as 3-*O*-caffeoylquinic acid.[49]

The triterpenes lupeol (**1**) and β-amyrin (**3**) and the flavones velutin (**10**), apigenin (**11**) and chrysoeriol (**12**) were previously isolated from *V. nudiflora*.[23] Despites the sesquiterpene lactones piptocarphin A (**6**), B (**7**) and D (**8**) already described in other *Vernonanthura* and *Vernonia* species,[50-53] this is the first report of piptocarphins in *V. nudiflora*. Also, this is the first description of the presence of **2**, **4**-**5** and **13**-**17** in *V. nudiflora* aerial parts besides the report of the new hirsutinolide sesquiterpene lactone **9**.

Structural elucidation of 8α-tigloyloxy-10α-hydroxy-hirsutinolide (**9**)

Compound **9** was obtained as a colorless gum, $[\alpha]_D^{24}$ = +26.6 in $CHCl_3$. The molecular formula was established as $C_{20}H_{26}O_8$, based on HR-ESI-MS (*m/z* 417.1525 $[M + Na]^+$, calcd. 417.1526). The ^{1}H and ^{13}C NMR spectra (Table 1) were very similar to those reported for piptocarphins A (**6**), B (**7**) and D (**8**)[40] and as it is characteristic for hirsutinolides derivatives, the ^{1}H NMR spectrum exhibited broad signals.[41]

The ^{13}C NMR signals at δ_C 166.9 (C-12), 149.7 (C-7), 144.2 (C-6) and 128.1 (C-11) indicate the presence of a γ-lactone group. Also, an oxygenated quaternary carbon at δ_C 82.0 (C-4) and a ketal quaternary carbon at δ_C 108.6 (C-1) were observed. The ^{1}H NMR and HSQC spectra revealed two methyl groups at [δ_H 1.25 (s) / δ_C 25.4 (C-14) and δ_H 1.57 (s) / δ_C 29.7 (C-15)], four methylenes at [δ_H 1.94-1.96 (m) / δ_C 31.9 (C-2), δ_H 2.40-2.46 (m) / δ_C 31.9 (C-3), δ_H 2.61 (dd, *J* 11.0, 15.6 Hz, H-9β), 2.12-2.15 (m, H-9α) / δ_C 38.1 (C-9), and [δ_H 4.62 (m), 4.60 (m) / δ_C 54.6 (C-13)], an oxymethine at δ_H 6.60 (brd, *J* 11.5 Hz) / δ_C 65.7 (C-8), and an olefinic signal at δ_H 5.87 (s)/δ_C 126.0 (C-5), all these signals corroborate the skeleton of a hirsutinolide-type sesquiterpene.[40,42,51] In addition, the ^{1}H and ^{13}C NMR spectra showed an olefinic signal at δ_H 7.06 (q, *J* 7.5 Hz) / δ_C 140.3 (C-3'), two methyl groups downfield at δ_H 1.84 (d, *J* 7.6 Hz) / δ_C 11.9 (C-4') and δ_H 1.82 (s) / δ_C 14.7 (C-5'), an olefinic quaternary carbon at δ_C 128.2 (C-2'), and an ester carbonyl carbon at δ_C 166.8 (C-1'), that were indicative of a tigloyl moiety.

The HMBC spectrum showed correlations between of the methyl groups H-14 (δ_H 1.25) with C-1 (δ_C 108.6), C-10 (δ_C 78.1), C-9 (δ_C 38.1) and H-15 (δ_H 1.57) with C-4 (δ_C 82.0), C-3 (δ_C 37.6), and C-5 (δ_C 126.0). Furthermore, the correlation of H-3 (δ_H 2.40-2.46) with C-2 (δ_C 31.9) permitted to evidence the presence of the cyclic ether in the structure. In addition, the correlations of H-5' (δ_H 1.82) with C-1' (δ_C 166.9), C-2' (δ_C 128.1) and C-3' (δ_C 140.3) permitted assignment of the tigloyl group in the structure (Figure 2). The attachment of this group at C-8 in compound **9** was supported by the NMR chemical shifts values of H-8 reported for hirsutinolides esterified in the C-8 position (δ_H 6.36 to 6.60)[40,42] and non-esterified (δ_H 5.46).[40,54] The signal for H-8 of compound **9** appears in δ_H 6.60, which is consistent with the linkage of tigloyloxy group at C-8.

NOESY spectra showed correlations between the hydroxyl groups at δ_H 4.11 (OH-1) and δ_H 3.88 (OH-10) with the methyl group at δ_H 1.57 (CH_3-15). Besides that, correlations of H-8 (δ_H 6.60) with H-9β (δ_H 2.61), and between H-9β and methyl group CH_3-14 (δ_H 1.25), indicated that H-8 is in the β position (Figure 2). Moreover, previous studies have reported that 8*S** configuration appears to be generally the most accepted for hirsutinolides-type with H-8 in the β position.[41,42,51]

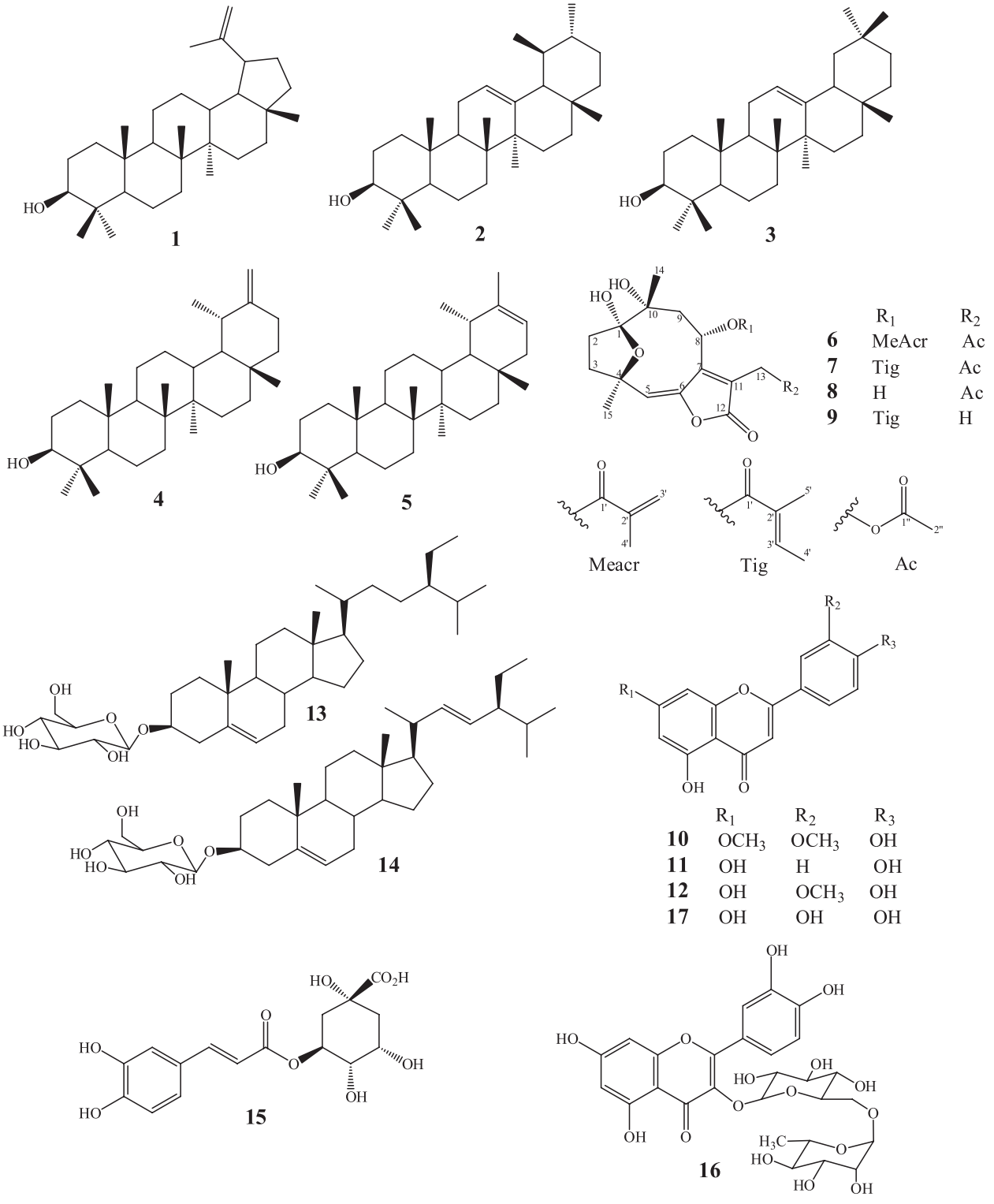

Figure 1. Chemical structures of the compounds isolated from *V. nudiflora.*

Identification of sesquiterpene lactones in the dichloromethane fraction by UHPLC-HRMS/MS

The dichloromethane fraction (DC) of *V. nudiflora* was also analyzed by UHPLC-HRMS/MS (positive ion mode), using the sesquiterpene lactones **6-9,** previously isolated and characterized by NMR analysis as standards. This strategy allowed the identification of more nine sesquiterpene lactones (Figure 3, Table 2).

First, we analyzed the fragmentation profile of compounds

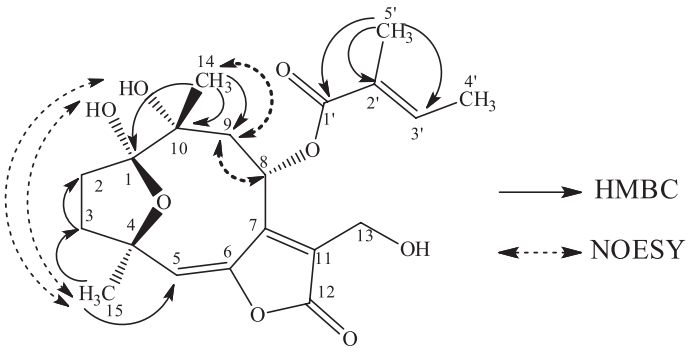

Figure 2. Main correlations observed in the HMBC and NOESY spectrum for compound **9**.

Table 1. ^{1}H and ^{13}C NMR data for compounds **6**, **7**, **8** and **9** in $CDCl_3$

Position	**6**[a]		**7**[a]		**8**[a]		**9**[b]	
	δ_H / ppm (*J* / Hz)	δ_C / ppm	δ_H / ppm (*J* / Hz)	δ_C / ppm	δ_H / ppm (*J* / Hz)	δ_C / ppm	δ_H / ppm (*J* / Hz)	δ_C / ppm
1	–	108.6	–	108.6	–	110.6	–	108.6
2	1.95 m	31.9	1.95 m	31.9	1.96 m	32.4	1.94-1.96 m	31.9
	1.84 m		1.84 m					
3	2.62 m	37.4	2.62 m	37.4	2.45 m	37.5	2.40-2.46 m	37.6
4	–	82.1	–	82.1	–	82.8	–	82.0
5	5.89 s	126.8	5.89 s	126.8	5.88 s	124.8	5.87 brs	126.0
6	–	144.0	–	144.0	–	143.6	–	144.2
7	–	150.0	–	150.0	–	154.7	–	149.7
8	6.60 brd (7.5)	66.1	6.60 brd (7.5)	66.1	5.46 ddd (13.0; 10.0; 2.0)	64.1	6.60 brd (7.5)	65.7
9β	2.62 m	38.0	2.62 m	38.0	2.53 dd (10.8;15.7)	38.1	2.61 dd (11.0;15.6)	38.1
9α	2.12 m		2.12 m		2.13-2.18 m		2.12-2.15 m	
10	–	78.1	–	78.1	–	77.2	–	78.1
11	–	131.0	–	131.0	–	126.6	–	128.1
12	–	166.9	–	166.9	–	166.7	–	166.9
13a	5.33 d (12.5)	55.7	5.33 d (12.5)	55.7	4.96 d (13.0)	53.7	4.62 m	54.6
13b	4.92 d (12.5)		4.92 d (12.5)		4.84 d (13.0)		4.60 m	
14	1.24 s	25.5	1.24 s	25.5	1.23 s	26.2	1.25 s	25.4
15	1.54 s	29.0	1.54 s	29.0	1.66 s	29.3	1.57 s	29.7
1’	–	165.8	–	166.8	–	–	–	166.8
2’	–	135.8	–	127.3	–	–	–	128.2
3a’	6.28 brs	127.3	7.05 m	139.7	–	–	7.05 q (7.5)	140.3
3b’	5.69 m							
4’	1.95 brs	18.1	1.84 d (5.5)	11.9	–	–	1.85 d (7.6)	11.9
5’	–	–	1.83 s	14.6	–	–	1.82 s	14.7
1”	–	170.4	–	170.4	–	170.3	–	–
2”	2.08 s	20.7	2.08 s	20.7	2.09 s	20.9	–	–
1–OH	4.13 brs	–	4.13 brs	–	4.80 brs	–	4.11 brs	–
10–OH	3.85 brs	–	3.85 brs	–	4.51 d (2.5)	–	3.88 brs	–
8–OH	–	–	–	–	6.12 d (13.0)	–	–	–

[a]^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz); [b]^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz); δ: chemical shift; *J*: coupling constant; s: singlet; d: doublet; dd: doublet of doublets; ddd: doublet of doublet of doublets; m: multiplet; q: quartet; brs: broad singlet; brd: broad doublet.

6-**9**. For compound **9** ($[M + Na]^+$ *m/z* 417.1525), the main fragments were at *m/z* 399.1417 ($[M + Na - (H_2O)]^+$, 335.1118 ($[M + Na - (C_5H_6O)]^+$), 317.0998 (base peak, $[M + Na - (C_5H_8O_2)]^+$) and 299.0892 (base peak, $[M + Na - (C_5H_8O_2) - (H_2O)]^+$). The compounds **6** ($[M + Na]^+$ *m/z* 445.1477) and **7** ($[M + Na]^+$ *m/z* 459.1633) showed a similar fragmentation profile with a base ion peak produced by the loss of methacrylate ($C_4H_6O_2$) and tiglate ($C_5H_8O_2$) groups, respectively. Finally, compound **8** ($[M + H - (H_2O)]^+$) *m/z* 337.1285) exhibited a base ion peak at *m/z* 259.0965 corresponding to the loss of acetyl group (C_2H_3O) and successive eliminations of two molecules of H_2O.

With this information, and based on literature data concerning to sesquiterpene lactones of *Vernonanthura*, we were able to propose the putative identification of seven hirsutinolide-type (**18**-**24**) and two glaucolide-type (**25**-**26**) (Figure 3, Table 2). The identification was established on the basis of the fragmentation pattern of their main ion by MS/MS analysis, and compared to those already described in literature from hirsutinolides.[40,55-57] Compounds **18**-**22** and **25**-**26** were previously reported from *V. nudiflora*,[23] whereas compounds **23** and **24** are being described herein for the first time for this species.

Antiproliferative activity

The antiproliferative activity of the crude extract, fractions and isolated compounds of *V. nudiflora* aerial parts were described as GI_{50}, TGI and SI values (Tables 3 and 4). To analyze these results, we assumed the criteria described by Fouche *et al.*[58] that stablished the values of $GI_{50} \leq 30$ μg mL^{-1} and TGI ≤ 50 μg mL^{-1} for promisor antiproliferative samples.

Considering these criteria, the crude extract (CE) of *V. nudiflora* was able to inhibit 50% of growth of almost all cell lines tested, being more selective against glioma (U251, GI_{50} = 2.13 μg mL^{-1}, SI = 11.9, TGI = 34.59 μg mL^{-1}). Among the fractions, hydromethanolic fraction (HM) was inactive (GI_{50} > 30 μg mL^{-1} and TGI > 50 μg mL^{-1}) for all cell lines while butanolic fraction (BU) was active only for ovarian tumor cells (OVCAR-03, GI_{50} = 11.16 μg mL^{-1}). The ethyl acetate fraction (EA) showed a moderate cytostatic effect against leukemia (K562, GI_{50} 1.19 μg mL^{-1}; SI = 22.3) and ovarian (OVCAR-03, GI_{50} 4.12 μg mL^{-1}; SI = 6.4) tumor cell lines. From this fraction, we isolated apigenin (**11**), crysoeriol (**12**), 3-*O*-caffeoylquinic acid (**15**), rutin (**16**) and luteolin (**17**). Many studies have demonstrated that polyphenols, including flavonoids, can act at different points during the carcinogenesis process, through several mechanisms of action, alone or in a synergistic way.[59,60]

The dichloromethane fraction (DC) inhibited all human tumor and non-tumor cell lines tested with GI_{50} and TGI values ranging from 0.27 to 3.98 μg mL^{-1} and 1.60 to 29.37 μg mL^{-1}, respectively, being selective for

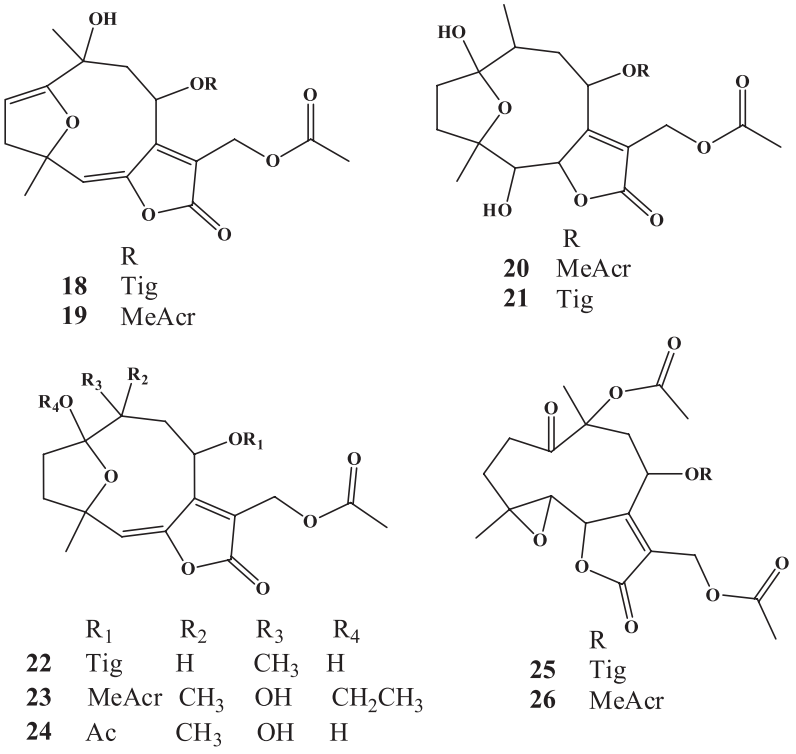

Figure 3. Chemical structures of the sesquiterpene lactones identified in the dichloromethane fraction by UHPLC-HRMS/MS.

Table 2. Data of the compounds identified in the dichloromethane fraction of *V. nudiflora* by UHPLC-HRMS/MS

Compound	Molecular formula	Exact mass *m/z*	Precursor ion *m/z*	Mass error / ppm	t_R / min	Fragment ions
6[a]	$C_{21}H_{26}O_9$ $[M + Na]^+$	445.1475	445.1477	0.45	4.75	359; 299; 259; 241; 213
6[b]	$C_{21}H_{26}O_9$ $[M + Na]^+$		445.1470[55]			385; 359; 277; 299; 259; 241; 213[55]
7[a]	$C_{21}H_{28}O_9$ $[M + Na]^+$	459.1631	459.1633	0.43	4.85	377; 359; 299; 259; 241; 213
7[b]	$C_{21}H_{28}O_9$ $[M]^{\bullet+}$		433.1760[40]			401; 377; 359; 337; 319; 301; 277; 259[40]
8[a]	$C_{17}H_{22}O_8[M - H_2O]^+$	337.1282	337.1284	0.59	4.34	319; 303; 277; 259; 241; 217
8[b]	$C_{17}H_{22}O$ $[M - H_2O]^+$		337.1273[55]			319; 277; 259; 241; 235; 231; 217[55]
9[a]	$C_{20}H_{26}O_8$ $[M + Na]^+$	417.1526	417.1525	0.23	4.48	399; 359; 317; 295; 259; 241; 213
18[a]	$C_{22}H_{26}O_8$ $[M + H]^+$	419.1700	419.1707	1.67	4.86	359; 319; 277; 259; 241; 231; 213
19[a]	$C_{21}H_{28}O_8[M + Na]^+$	427.1369	427.1368	0.23	4.82	385; 367; 321; 299; 277; 259; 231; 213
20[a]	$C_{21}H_{28}O_9$ $[M + Na]^+$	447.1630	447.1634	0.89	4.95	387; 359; 327; 301; 277; 259; 241; 231; 213
21[a]	$C_{22}H_{30}O_9$ $[M + Na]^+$	461.1787	461.1812	5.42	5.08	399; 373; 359; 345; 301; 277; 259; 241; 231; 213
22[a]	$C_{22}H_{28}O_8$ $[M + Na]^+$	443.1682	443.1682	0	5.05	383; 343; 301; 299; 283; 261
22[b]	$C_{22}H_{28}O_8$ $[M]^{\bullet+}$		420.1780[56]			403; 360; 260; 242[56]
23[a]	$C_{23}H_{30}O_9$ $[M + Na]^+$	473.1787	473.1795	1.69	5.26	431; 413; 387; 373; 345; 327; 313; 285; 259; 231; 217
23[b]	$C_{23}H_{30}O_9$ $[M]^{\bullet+}$		450.1860[40]			405; 345; 319; 301; 259[40]
24[a]	$C_{19}H_{24}O_9$ $[M + Na]^+$	419.1318	419.1320	0.47	4.42	359; 299; 259; 241; 231
24[b]	$C_{19}H_{24}O_9$ $[M + Na]^+$		419.1318[55]			359; 319; 299; 277; 259; 241; 231[55]
25[a]	$C_{24}H_{30}O_{10}$ $[M + Na]^+$	501.1737	501.1739	0.40	5.12	459; 441; 401; 385; 359; 341; 319; 281; 259; 241; 217
25[b]	$C_{24}H_{30}O_{10}$ $[M + H]^+$		479.00[57]			419; 379; 359; 321; 277; 261; 259[57]
26[a]	$C_{23}H_{28}O_{10}$ $[M+H]^+$	465.1755	465.1762	1.50	5.01	405; 379; 345; 319; 277; 259; 241; 231; 213

[a]Experimental data; [b]literature data; t_R: retention time; fragment ions from compounds **18**-**22** and **26** were not found.

leukemia (K562, GI_{50} = 0.27 μg mL^{-1}, SI = 3.7) and kidney (786-0, TGI = 1.60 μg mL^{-1}) tumor cell lines. In this fraction, we identified the sesquiterpene lactones of the hirsutinolide class (**6**-**9** and **18**-**24**), the flavonoids velutin (**10**), apigenin (**11**), chrysoeriol (**12**) and the mixture of sitosterol-3-β-*O*-*D*-glicopyranoside and stigmasterol-3-β-*O*-*D*-glicopyranoside (**13** + **14**).

Besides the description of antiproliferative properties for many flavonoids and for the steroidal mixture **13** + **14**,[61,62] previous studies have reported a high cytotoxic activity for hirsutinolides against different tumor cells.[27,53,63-65] Therefore, the high antiproliferative potential observed for DC fraction can be partially justified by the presence of hirsutinolide sesquiterpene lactones, toghether with flavonoids and steroids that in association may have contributed to final effect.

Due to the limited sample amounts, in this work we were able to evaluate the antiproliferative activity of the mixture of piptocarphins A and B (**6** + **7**), piptocarphin D (**8**), the new sesquiterpene lactone **9** and the flavonoid velutin (**10**) (Tables 3 and 4). The mixture of piptocarphins A and B (**6** + **7**) showed strong activity against all human cell lines tested ($GI_{50} \leq 0.15$ μg mL^{-1}, TGI from 2.50 to 25.10 μg mL^{-1}) corroborating the previous description of piptocarphin A effect against leukemic cell line HL-60 (IC_{50} = 3.87 μmol L^{-1}).[53] Piptocarphin D (**8**) presented promising results being selective against resistant ovarian (NCI-ADR/RES, GI_{50} = 0.15 μg mL^{-1}, SI = 24.7, TGI = 7.80 μg mL^{-1}) and glioma (U251, GI_{50} = 1.5 μg mL^{-1}, SI = 2.46, TGI = 12.50 μg mL^{-1}) cell lines. The new hirsutinolide-type derivative, 8α-tigloyloxy-10α-hydroxy-hirsutinolide (**9**), showed potent activity mainly against breast (MCF-7, GI_{50} = 0.96 μg mL^{-1}, SI = 5.7, TGI = 18.60) and resistant ovarian (NCI-ADR/RES, GI_{50} = 3.60 μg mL^{-1}, SI = 5.7) cell lines. Considering the chemical structures of compounds **6**-**9** (Figure 1), it seems that the substituent groups at C-8 and C-13 may modulate the selectivity of these compounds. Velutin (**10**) showed cytostatic activity against breast (MCF-7, GI_{50} = 1.40 μg mL^{-1}, SI = 2.1) and lung (NCI-H460, GI_{50} = 3.70 μg mL^{-1}, SI = 0.8) tumor cell lines.

Finally, the hexane fraction (HE) showed a moderate cytostatic activity against almost all cell lines tested, being more effective for glioma (U251, GI_{50} = 2.36 μg mL^{-1}, SI = 4.6, TGI = 36.80 μg mL^{-1}) and leukemia (K562, GI_{50} = 5.21 μg mL^{-1}; SI = 2.1, TGI = 37.03 μg mL^{-1}). HE fraction was found to contain the pentacyclic triterpenes

lupeol (**1**), α-amyrin (**2**), β-amyrin (**3**), taraxasterol (**4**) and pseudotaraxasterol (**5**), besides the mixture of **6** and **7**. According to the literature, lupane-type pentacyclic triterpenes have usually been described as promising multitarget antitumor agents.[66,67]

Antioxidant activity

The scavenging ability, assessed by the DPPH and ABTS methods, together with the TPC and TF content allowed the evaluation of antioxidant activity of crude extract and fractions of *V. nudiflora* aerial parts (Table 5).

Considering of the ABTS and DPPH assays, the polar EA (IC_{50} = 13.09 μg mL^{-1} for DPPH and 4.80 μg mL^{-1} for ABTS) and BU (IC_{50} = 15.10 μg ml^{-1} for DPPH and 5.90 μg mL^{-1} for ABTS) fractions exhibited higher scavenging potential than the non-polar fractions (HE and DC). These results suggested that antioxidant compounds present in crude extract (CE) were concentrated on these two fractions. Moreover, both EA and BU fractions showed IC_{50} values similar to the positive controls tested (rutin: 10.74 μg mL^{-1} and trolox: 2.59 μg mL^{-1}).

Regarding the TPC and TF content, as expected, EA (526.80 mg EAG g^{-1}; 97.13 mg EQ g^{-1}) and BU (426.19 mg EAG g^{-1}; 82.07 mg EQ g^{-1}) fractions presents

Table 3. Antiproliferative activity of the crude extract, fractions and some compounds isolated from *V. nudiflora* aerial parts

	GI_{50} / (μg mL^{-1})										
	2[a]	u[a]	m[a]	a[a]	7[a]	4[a]	p[a]	o[a]	h[a]	k[a]	q[b]
CE	2.13	27.62	26.46	41.12	22.35	29.28	35.78	25.64	30.45	17.51	25.26
HE	2.36	27.25	25.08	27.4	26.29	30.11	27.92	27.80	233.01	5.21	10.81
DC	0.66	2.75	1.50	1.42	1.24	2.18	3.98	0.78	2.26	0.27	1.00
EA	27.06	76.30	29.71	52.13	63.17	44.78	29.21	4.12	49.54	1.19	26.50
BU	[c]	[c]	[c]	[c]	[c]	[c]	[c]	11.16	[c]	67.11	[c]
HM	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]
Doxorubicin[d]	< 0.025	< 0.025	< 0.025	0.07	< 0.025	< 0.025	0.09	0.08	0.10	0.11	0.03
6 + 7	< 0.15	n.t.	< 0.15	0.15	n.t.	< 0.15	< 0.15	n.t.	< 0.15	n.t.	< 0.15
8	1.50	n.t.	6.60	0.15	n.t.	8.40	3.80	n.t.	8.10	n.t.	3.70
9	7.80	n.t.	0.96	3.60	n.t.	7.90	14.80	n.t.	12.00	n.t.	5.50
10	12.60	n.t.	1.40	[c]	n.t.	3.70	16.30	n.t.	26.30	n.t.	3.00
Doxorubicin[e]	< 0.025	n.t.	< 0.025	0.03	n.t.	< 0.025	0.103	n.t.	0.097	n.t.	< 0.025
	TGI / (μg mL^{-1})										
	2[a]	u[a]	m[a]	a[a]	7[a]	4[a]	p[a]	o[a]	h[a]	k[a]	q[b]
CE	34.59	119.33	163.09	[c]	[c]	98.18	199.55	200.89	210.36	55.72	247.76
HE	36.80	80.55	83.41	120.99	118.87	131.74	101.10	167.66	[c]	37.03	50.54
DC	9.64	19.28	14.02	29.37	1.60	11.37	22.10	22.07	22.05	8.10	[c]
EA	126.34	[c]	114.09	[c]	[c]	[c]	231.74	[c]	[c]	54.85	[c]
BU	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]
HM	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]
Doxorubicin[d]	0.16	0.079	[c]	8.76	0.62	6.96	[c]	[c]	4.16	1.83	6.86
6 + 7	3.70	n.t.	10.80	12.90	n.t.	2.50	25.10	n.t.	6.50	n.t.	2.90
8	12.50	n.t.	15.00	7.80	n.t.	46.90	36.10	n.t.	48.90	n.t.	36.90
9	21.20	n.t.	18.60	75.00	n.t.	24.00	[c]	n.t.	[c]	n.t.	[c]
10	[c]	n.t.	[c]	[c]	n.t.	[c]	[c]	n.t.	[c]	n.t.	[c]
Doxorubicin[e]	0.25	n.t.	0.37	1.77	n.t.	0.59	1.59	n.t.	11.45	n.t.	0.31

[a]Human tumor cell lines: 2: U251 (glioma); u: UACC-62 (melanoma); m: MCF-7 (breast); a: NCI-ADR/RES (ovarian with multiple drug resistance phenotype); 7: 786-0 (kidney); 4: NCI-H460 (kidney, non-small cell type), p: PC-3 (prostate), o: OVCAR-03 (ovarian), h: HT-29 (colon), k: K562 (leukemia); [b]human non-tumor cell line: q: HaCaT (keratinocyte); [c]effective concentration higher than the higher tested concentration; [d,e]doxorubicin: reference chemotherapy [first (d) and second (e) experiments]; n.t.: not tested; GI_{50}: growth inhibition 50 - concentration required to inhibit 50% of cell growth; TGI: total growth inhibition; CE: crude extract; HE: hexane fraction; DC: dichloromethane fraction; EA: ethyl acetate fraction; BU: butanolic fraction; HM: hydromethanolic fraction; **6 + 7**: piptocarphin A and B; **8**: piptocarphin D; **9**: 8α-tigloyloxy-10α-hydroxy-hirsutinolide; **10**: velutin.

Table 4. Selectivity index for antiproliferative activity of the crude extract, fractions and some compounds isolated from *V. nudiflora* aerial parts

	SI[a]									
	2[b]	u[b]	m[b]	a[b]	7[b]	4[b]	p[b]	o[b]	h[b]	k[b]
CE	11.9	0.9	1.0	0.6	1.1	0.9	0.7	1.0	0.8	1.4
HE	4.6	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.0	2.1
DC	1.5	0.4	0.7	0.7	0.8	0.5	0.3	1.3	0.4	3.7
EA	1.0	0.3	0.9	0.5	0.4	0.6	0.9	6.4	0.5	22.3
BU	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]
HM	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]
Doxorubicin[d]	[c]	[c]	[c]	0.4	[c]	[c]	0.3	0.4	0.3	0.3
6+7	[c]	n.t.	[c]	[c]	n.t.	[c]	[c]	n.t.	[c]	n.t.
8	2.5	n.t.	0.6	24.7	n.t.	0.4	1.0	n.t.	0.5	n.t.
9	0.7	n.t.	5.7	1.5	n.t.	0.7	0.4	n.t.	0.5	n.t.
10	0.2	n.t.	2.1	[c]	n.t.	0.8	0.2	n.t.	0.1	n.t.
Doxorubicin[e]	[c]	n.t.	[c]	[c]	n.t.	[c]	[c]	n.t.	[c]	n.t.

[a]SI: selectivity index (SI = GI_{50} HaCat / GI_{50} tumor cell line); [b]human tumor cell lines: 2: U251 (glioma); u: UACC-62 (melanoma); m: MCF-7 (breast); a: NCI-ADR/RES (ovarian with multiple drug resistance phenotype); 7: 786-0 (kidney); 4: NCI-H460 (kidney, non-small cell type), p: PC-3 (prostate), o: OVCAR-03 (ovarian), h: HT-29 (colon), k: K562 (leukemia); [c]not calculated (GI_{50} HaCat and/or GI_{50} tumor cell line was above or below the higher or the lower tested concentration, respectively); [d,e]doxorubicin: reference chemotherapy [first (d) and second (e) experiments]; n.t.: not tested; CE: crude extract; HE: hexane fraction; DC: dichloromethane fraction; EA: ethyl acetate fraction; BU: butanolic fraction; HM: hydromethanolic fraction; **6 + 7**: piptocarphin A and B; **8**: piptocarphin D; **9**: 8α-tigloyloxy-10α-hydroxy-hirsutinolide; **10**: velutin.

Table 5. Antioxidant activity by DPPH and ABTS assays, and total phenolic compounds (TPC) and total flavonoids (TF) for the crude extract and fractions of the aerial parts of *V. nudiflora*

Samples	DPPH IC_{50} / (μg mL^{-1})	ABTS IC_{50} / (μg mL^{-1})	TPC / (mg GAE g^{-1})	TF / (mg QE g^{-1})
CE	35.32 ± 0.12[d]	15.85 ± 0.02[b]	113.20 ± 0.14[d]	78.05 ± 0.89[c]
HE	187.71 ± 0.94[a]	n.a.	9.80 ± 0.18[f]	36.25 ± 0.34[e]
DC	96.75 ± 0.67[b]	22.87 ± 0.40[a]	56.15 ± 0.31[e]	42.51 ± 0.46[d]
EA	13.09 ± 0.19[f]	4.80 ± 0.02[e]	526.80 ± 1.35[a]	97.13 ± 4.85[a]
BU	15.10 ± 0.31[e]	5.90 ± 0.03[d]	426.19 ± 2.10[b]	82.07 ± 6.86[b]
HM	46.64 ± 1.71[c]	12.42 ± 0.11[c]	177.27 ± 0.97[c]	22.44 ± 5.64[f]
Rutin	10.74 ± 0.12[g]	n.t.	n.t.	n.t.
Ascorbic acid	3.60 ± 0.09[h]	n.t.	n.t.	n.t.
Trolox	n.t.	2.59 ± 0.01[f]	n.t.	n.t.

Data are expressed as the mean ± standard deviation. Within a column, values with the same superscript letters are not significantly different from each other ($p < 0.05$); n.a: not active; n.t.: not tested. DPPH:1,1-diphenyl-2-picrylhydrazyl; ABTS: (2,2-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid; GAE: gallic acid equivalents; QE: quercetin equivalent; CE: crude extract; HE: hexane fraction; DC: dichloromethane fraction; EA: ethyl acetate fraction; BU: butanolic fraction; HM: hydromethanolic fraction.

the higher levels of phenols and flavonoids than HE and DC fractions. Interestingly, the most polar hydromethanolic fraction (HM) presented lower antioxidant activity, probably due to the grouping of all antioxidant compounds in EA and BU fractions in view of the exhaustive partition process.

In the present study, phenolic compounds (**11, 12**, **15**, **16** and **17**) were isolated from the EA fraction of *V. nudiflora* aerial parts (Figure 1). These phenolic compounds, in particular rutin, have been extensively studied because of their higth antioxidant activity and its relation with health.[49,68-70] Therefore, our results indicated the potential use of polar fractions of *V. nudiflora* as a natural antioxidant product.

Conclusions

In the present work we investigated the chemical composition, as well as the antioxidant potential and antiproliferative activity of *Vernonanthura nudiflora*

aerial parts. Besides 25 known compounds, some of them identified for the first time in *V. nudiflora*, one new hirsutinolide derivative was also described. These results contribute to phytochemical knowledge of this species, and suggest that sesquiterpene lactones, especially of hirsutinolide-type, can be considered chemotaxonomic markers of the *Vernonanthura* genus.

The polar fractions (EA and BU) showing higher content of phenolic compounds and flavonoids, presented higher antioxidant activity, with IC_{50} values similar to the positive controls. Furthermore, the non-polar fractions (HE and DC) showed the better antiproliferative activity that could be attributed to the presence of hirsutinolide sesquiterpene lactones. The cytostatic effects observed for some isolated sesquiterpene lactones supported this hypothesis. Concluding, these results showed the potential of *V. nudiflora* aerial parts as a promising source of bioactive molecules for a potential use of as a natural antioxidant, and in the treatment of different types of cancer.

Supplementary Information

Supplementary data of ^{1}H NMR, HSQC, HMBC, NOESY and HR-ESI-MS for compounds **6**-**9** and HR-ESI-MS/MS for compounds **18**-**26** are available free of charge at http://jbcs.sbq.org.br as a PDF file.

Acknowledgments

The authors would like to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) for financial support. This work was supported by the Complexo de Central de Apoio à Pesquisa (COMCAP) from the Universidade Estadual de Maringá (UEM).

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Submitted: December 9, 2018
Published online: April 30, 2019