



Saponins from the rhizomes of *Chamaecostus subsessilis* and their cytotoxic activity against HL60 human promyelocytic leukemia cells

[Saponinas de los rizomas de *Chamaecostus subsessilis* y su actividad citotóxica contra las células de leucemia promielocítica humana HL60]

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Abstract

Context: Species of the Costaceae family have been traditionally used for the treatment of infections, tumors and inflammatory diseases. *Chamaecostus subsessilis* (Nees & Mart.) C. D. Specht & D.W. Stev. (Costaceae) is a native medicinal plant with distribution in the Cerrado forest ecosystem of Central Brazil. In our previous work, the antitumor potential of the chloroform fraction (CHCl₃ Fr) from rhizomes ethanol extract (REEX) of *C. subsessilis* was determined against a series of tumor cell lines (HL60, Jurkat, MDA-MB231, MCF-7, HTC-116 and THP-1) using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric assay.

Aims: To isolate and identify the cytotoxic compounds present in CHCl₃ Fr from REEX of *C. subsessilis*.

Methods: The bio-guided chromatographic fractionation of the CHCl₃ Fr was performed by High-Performance Liquid Chromatography (HPLC) and preparative Thin Layer Chromatography (prep-TLC). The cytotoxicity of the isolated compounds was evaluated against human promyelocytic leukemia cells (HL60) and human embryonic renal non-tumor cell lineage (HEK-293) by MTT assay.

Results: Dioscin and gracillin were identified as active compounds from CHCl₃ Fr from REEX of *C. subsessilis*. They showed IC₅₀ values of 5.3 μM and 14.0 μM, respectively, against HL60 cells, but they did not demonstrate selectivity compared to HEK-293 cells.

Conclusions: These results corroborate with the popular use of species of the Costaceae family as a source of antitumor agents. To the best of our knowledge, this is the first time that these saponins are isolated from this species.

Keywords: *Chamaecostus subsessilis*; Costaceae; cytotoxic activity; saponins; tumor cell line.

Resumen

Contexto: Las especies de la familia Costaceae se han utilizado tradicionalmente para el tratamiento de infecciones, tumores y enfermedades inflamatorias. *Chamaecostus subsessilis* (Nees y Mart.) C. D. Specht y D.W. Stev. (Costaceae) es una planta medicinal nativa con distribución en el ecosistema forestal Cerrado del centro de Brasil. En nuestro trabajo anterior, el potencial antitumoral de la fracción de cloroformo (CHCl₃ Fr) del extracto de etanol de rizomas (REEX) de *C. subsessilis* se determinó frente a una serie de líneas celulares tumorales (HL60, Jurkat, MDA-MB231, MCF-7, HTC -116 y THP-1) utilizando el ensayo colorimétrico basado en bromuro de 3-(4, 5-dimetiltiazol-2-il) -2, 5-difeniltetrazolio (MTT).

Objetivos: Aislar e identificar los compuestos citotóxicos presentes en CHCl₃ Fr de REEX de *C. subsessilis*.

Métodos: El fraccionamiento cromatográfico bioguiado del CHCl₃ Fr se realizó mediante cromatografía líquida de alta resolución (HPLC) y cromatografía preparativa de capa fina (prep-CCF). La citotoxicidad de los compuestos aislados se evaluó frente a las células de leucemia promielocítica humana (HL60) y el linaje de células no tumorales renales embrionarias humanas (HEK-293) mediante el ensayo MTT.

Resultados: Dioscina y gracillin se identificaron como compuestos activos de CHCl₃ Fr de REEX de *C. subsessilis*. Ellos mostraron valores de CI₅₀ de 5,3 μM y 14,0 μM, respectivamente, contra células HL60, pero no demostraron selectividad en comparación con las células HEK-293.

Conclusiones: Estos resultados corroboran con el uso popular de especies de la familia Costaceae como fuente de agentes antitumorales. Hasta donde sabemos, esta es la primera vez que estas saponinas se aíslan de esta especie.

Palabras Clave: actividad citotóxica; *Chamaecostus subsessilis*; Costaceae; línea celular tumoral; saponinas.

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INTRODUCTION

Several plants have been sources for the discovery of new drugs since they produce structurally complex compounds and can have high specificity for therapeutic targets (Newman and Cragg, 2016). Our research group has been driving to seek compounds of natural origin with activity against cancer. In our previous work, we showed that the rhizomes ethanol extract (REEX) from *Chamaecostus subsessilis* had cytotoxic activity against the six cell lines (HL60, Jurkat, MDA-MB231, MCF-7, HTC-116 and THP-1) by the MTT colorimetric assay with IC₅₀ values varying from 8.2 to 53.8 µg/mL. The REEX also induced DNA fragmentation in HL-60, Jurkat and THP-1 cell lines at the concentration of 20 µg/mL with higher apoptotic activities than etoposide in these cell lines. In addition, the CHCl₃ Fr from REEX showed a highlighted cytotoxic activity with IC₅₀ value of 2.6 µg/mL against HL-60 cells (Siqueira et al., 2016).

The *Chamaecostus* genus has seven known species, all endemic from South America, among them *Chamaecostus subsessilis* (Nees & Mart.) C. D. Specht & D.W. Stev. (synonyms: *Costus subsessilis*, *Globba subsessilis*) is a native plant that has distribution in the Cerrado forest ecosystems of Central Brazil. This species is unusual ginger that has small stature (not exceeding 30 cm in height) with elliptical and adaxially strigose to glabrous leaves and belongs to the *Costaceae* family (*Zingiberales*) (André et al., 2015).

The roots and tubers of the several species of the *Costaceae* family have been traditionally used for the treatment of infections, tumors and inflammatory diseases (Pilla et al., 2006). It is known that some species from the *Costaceae* family, such as *Costus pictus* and *Costus speciosus* have cytotoxic properties (Jha et al., 2010; Nadumane et al., 2011; Sathuvan et al., 2012), but there are few studies of pharmacological properties of *Chamaecostus* genus. In this work, we describe the bioassay-guided purification of saponins responsible for the cytotoxicity of REEX.

MATERIAL AND METHODS

Materials and instruments

Semipreparative purifications were performed on an HPLC system (Shimadzu, Kyoto, Japan) coupled with an LC6AD pump (Shimadzu, Kyoto, Japan) and an SPD-10A-UV detector (Shimadzu, Kyoto, Japan), using a Shimpack® silica (5 µm, 20 × 250 mm, id) column. TLC analyses were conducted on pre-coated silica gel G-60/F₂₅₄ plates (0.25 mm, Merck, Darmstadt, Germany). The spots were visualized under UV light at 254 nm, 360 nm, visible, or by means of spraying the plates with a mixture of equal volumes of an ethanol solution of vanillin 1% w/v and sulfuric acid 10% v/v. The optical rotations were performed using an Anton Paar MCP 300 circular polarimeter (Anton Paar, Ashland, Virginia, USA). One- and two-dimensional nuclear magnetic resonance (NMR) experiments were run on a Bruker Avance 400 MHz spectrometer (Bruker Biospin, Rheinstetten, Baden-Württemberg, Germany), using tetramethylsilane as an internal standard. High-Resolution Mass Spectral (HRMS) data were measured using a Bruker maXis ESI-QTOF (Bruker Daltonics, Bremen, Germany) coupled to a Shimadzu Nexera UHPLC system (Kyoto, Japan) equipped with a Finnigan Surveyor PDA Plus diode-array detector. Dioscin (≥ 95%, HPLC) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Plant material

The fresh and healthy rhizomes of *C. subsessilis* were collected in the surrounding area of Zoo-Botanical Foundation (BHZZB, 19°51'31.0"S, 44°00'40.0"W) at Belo Horizonte, Minas Gerais, Brazil, in December 2013, under the governmental authorization Sisgen, receiving the number A83017A. The plant was identified by botanists of BHZZB since it was a species adapted to BHZZB area. A voucher specimen under the code BHZZB 8018 was deposited at BHZZB Herbarium.

Preparation of the crude ethanol extract and its fractions

Fresh rhizomes (748 g) were washed in water, dried at room temperature and chopped into small pieces of 1-5 cm. The slices were extracted three times with absolute ethanol (EtOH) at room temperature. The suspensions were filtered through a cellulose filter paper, and the EtOH was evaporated at 45°C under reduced pressure using a rotary evaporator. The residual solvent was removed in a vacuum centrifuge at 40°C to yield 7.3 g of extract. An aliquot of the crude ethanol extract (5 g) was dissolved in methanol: water (80:20 v/v) and sequentially partitioned (3 times) with solvents of increase polarity: hexane (HE), dichloromethane (CH₂Cl₂), chloroform (CHCl₃), ethyl acetate (EtOAc), and water (H₂O). The solvents were removed in a vacuum centrifuge at 40°C. This procedure yielded five fractions: HE (0.4 g), CH₂Cl₂ (1.1 g), CHCl₃ (2.0 g), EtOAc (0.4 g) and H₂O (3.0 g).

Bioassay-guided isolation of bioactive compounds

An aliquot of the CHCl₃ fraction (300 mg) was chromatographed on an HPLC system using silica column (250 × 20 mm, 5 μm, Shim-Pack®) and an isocratic elution with a mixture of dichloromethane: chloroform: methanol: water (4: 4: 3: 0.8) during 60 min. The eluent was pumped at 7 mL/min and the effluent absorption measured at λ 260 and 280 nm. One hundred and twenty fractions of 3.5 mL each were collected and pooled into 17 subfractions based on their TLC behavior after spraying the plates with a mixture of vanillin-sulfuric acid. Subfraction 4 showed cytotoxic activity against HL-60 at a concentration of 20 μg/mL (Fig. 1). It (100 mg) was purified by prep-TLC (silica gel G-60/F₂₅₄ plates, 0.50 mm) using a mixture of dichloromethane: chloroform: methanol: water (4:4:3:0.8) and eluted twice. This separation yielded the active compounds **1** (24 mg) and **2** (15 mg).

In vitro cytotoxicity assays

HL60 (human promyelocytic leukemia) and HEK-293 (human embryonic renal non-tumor) cell

lines were maintained in the logarithmic phase of growth in RPMI 1640 or rD-MEM medium supplemented with 100 IU/mL penicillin and 100 μg/mL streptomycin enriched with 2 mM of L-glutamine. Leukemia cells were cultured in RPMI and 10% fetal bovine serum. The cultures were maintained at 37°C in a humidified incubator with 5% CO₂ and 95% air. The media were changed twice weekly, and the cells were examined regularly. All cell lines were used for 20 passages.

The cell lines were inoculated using 5 × 10⁴ (HL60) or 1 × 10⁴ (HEK-293) cells per well. The plates were pre-incubated for 24 h at 37°C to allow the adaptation of cells prior to the addition of the test compounds. All samples were tested at a concentration of 20 μg/mL. The half-maximum inhibitory concentration (IC₅₀) of the isolated compounds was determined over a range of concentrations (eight nonserial dilutions from 100.0 to 0.6 μg/mL). Etoposide was evaluated under the same experimental conditions as positive control. All cell cultures were incubated in a 5% CO₂/95% air humidified atmosphere at 37°C for 48 h. The negative control included treatment with 0.5% (v/v) DMSO. Cell viability was estimated by measuring the rate of mitochondrial reduction after the addition of 10 μL of tetrazolium dye (MTT) at 2.5 mg/mL.

All the samples were tested in triplicate in three independent experiments. The results were expressed the mean of the percentage of viability in relation to the negative control (DMSO, 0.5% v/v), calculated as follows: percentage of cell viability (%) = [(OD of treated mean/OD negative control) × 100] (Mosmann, 1983).

All IC₅₀ values were calculated using the GraphPad Prism 7.0 statistical package (GraphPad Software, San Diego, CA, USA) according to the recommended protocol for nonlinear regression of a log (inhibitory) versus inverse response curve and were expressed as mean ± standard deviation (SD).

The selectivity index (SI) was defined as the ratio of the IC₅₀ value observed in HEK-293 cells to

the IC₅₀ value observed in the experiment with HL60 cell line for the same compound tested.

Statistical analysis

Results were expressed as mean ± standard deviation (SD) values of three independent experiments performed in the total of nine technical replicates. Statistical analysis was performed using the GraphPad Prism 7.0 statistical package (GraphPad Software, San Diego, CA, USA). Statistical differences were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons post-tests. Differences were considered to be significant at a limit of p<0.05.

RESULTS

The CHCl₃ Fr was chosen for chromatographic fractionation aiming the identification of active compounds since it demonstrated highlighted cytotoxic activity against HL60 cell line. The purification of the CHCl₃ Fr by normal-phase liquid chromatography yielded seventeen subfractions that were tested *in vitro* cytotoxicity assays against HL60 cells at a concentration of 20 µg/mL. Subfractions **2-4** showed the highest cytotoxic effect against HL60 cells with inhibition percentages above 90% (Fig. 1). These subfractions were analyzed by analytical TLC, showing a little complex profile and two main brown spots after revelation with vanillin/sulphuric acid. Preparative TLC of subfraction **4** yielded two compounds that revealed as brown spots after TLC analyses with the same condition of used for CHCl₃ Fr analysis.

The structures of the isolated compounds (**1** and **2**) were identified by a combination of spectroscopic methods, comparison with literature data and optical activities. The 1D and 2D data of spectra (Figs. 2 - 3) of isolated compounds were in accordance with the literature (Mahato, et al., 1980; Zou et al., 2003).

Compound **1** was obtained as a white amorphous powder. The HRMS displayed a protonated ion peak [M + H]⁺ at *m/z* 869.4907, under a molecular formula of C₄₅H₇₂O₁₆. The fragments ions at *m/z* 723.4318 [M+H-146]⁺, 577.3734 [M+H-146-146]⁺,

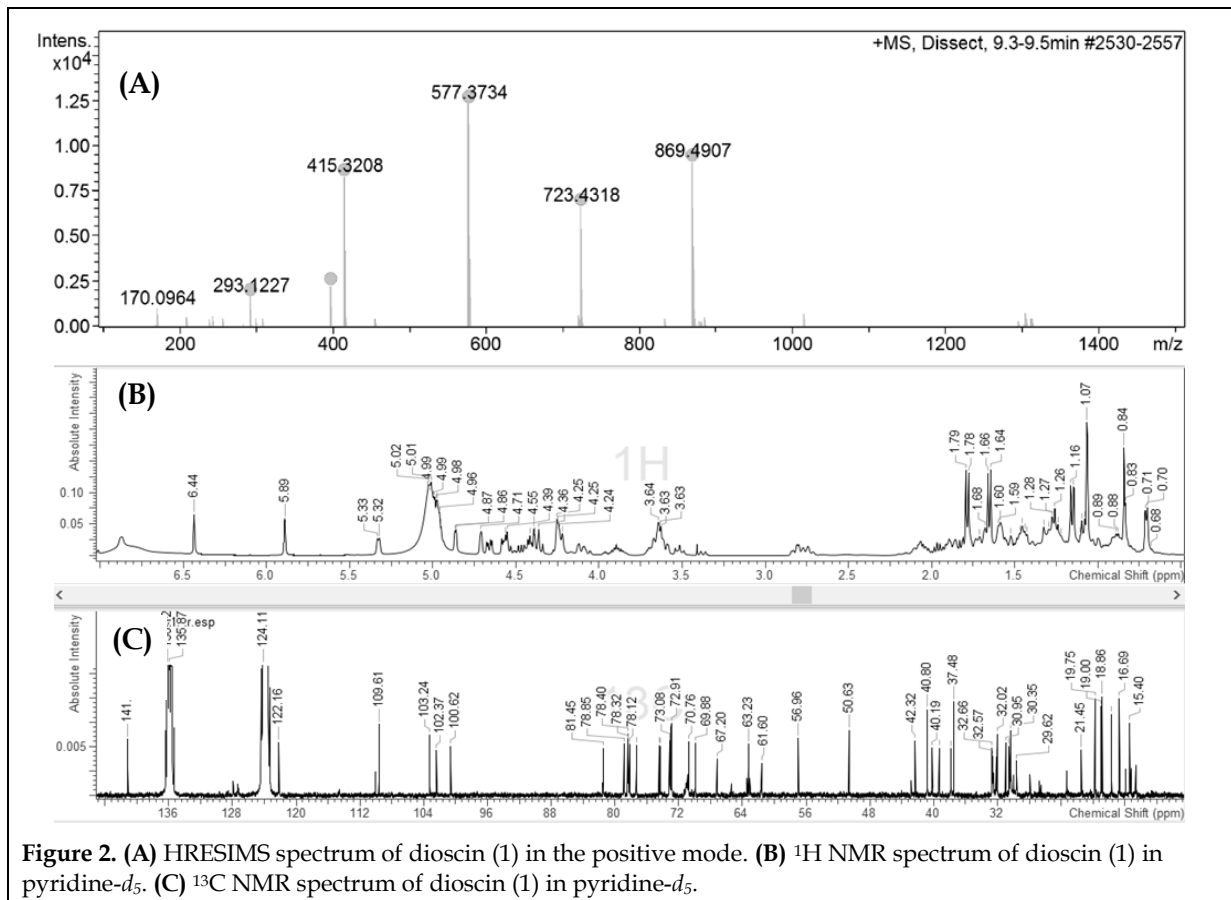
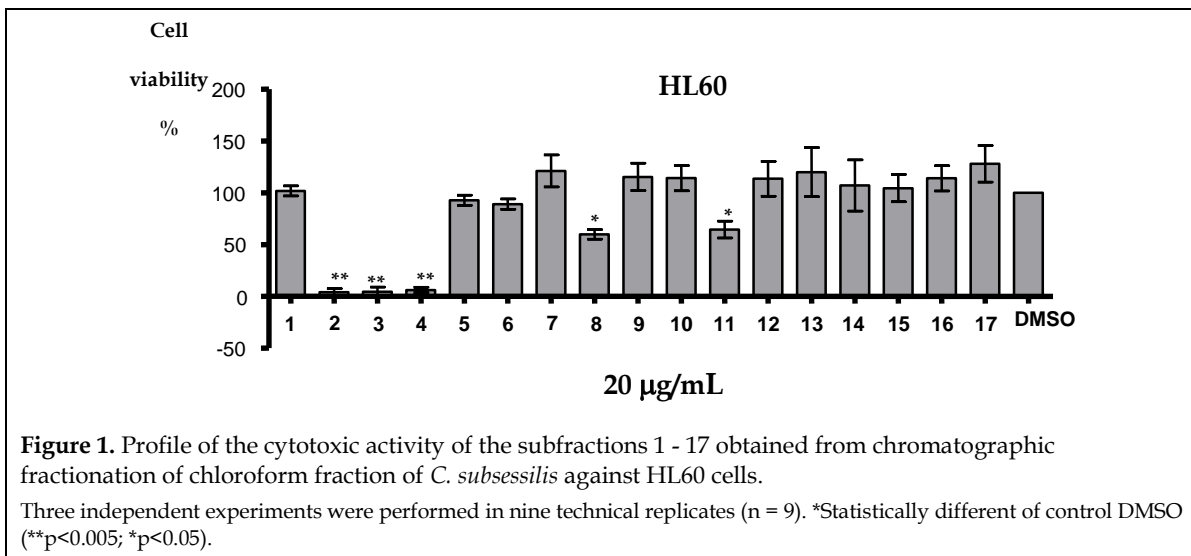
415.3208 [M+H-146-146-162]⁺ indicated the elimination of two deoxyhexoses and one hexose moieties, respectively. The presence of three sugar residues in compound **1** was confirmed by the HMQC experiment, where the anomeric protons at δ_H 6.44 (br, s), δ_H 5.89 (s) and δ_H 4.98 (overlapped signal), correlates with anomeric carbon signals at δ_C 102.4, δ_C 103.2 and δ_C 100.6 of α-rhamnopyranose, α-rhamnopyranose, and β-glucopyranose, respectively (Fig. 2). Compound **1** showed similar experimental optical activity [α]_D²⁵ = -99.4° (c 0.47, MeOH) to the literature [α]_D²⁵ = -102.2° (c 1.00, MeOH) (Pettit et al., 2005) for dioscin (diosgenin 3-O-α-L-rhamnopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside) (Fig. 4) and gave the same MS/MS fragmentation pattern when compared with an authentic sample by UHPLC-MS.

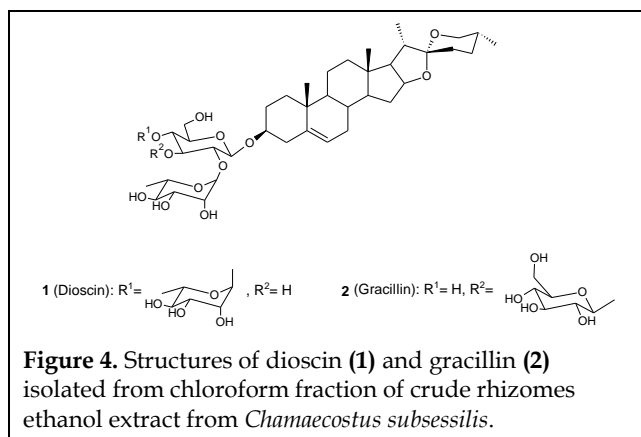
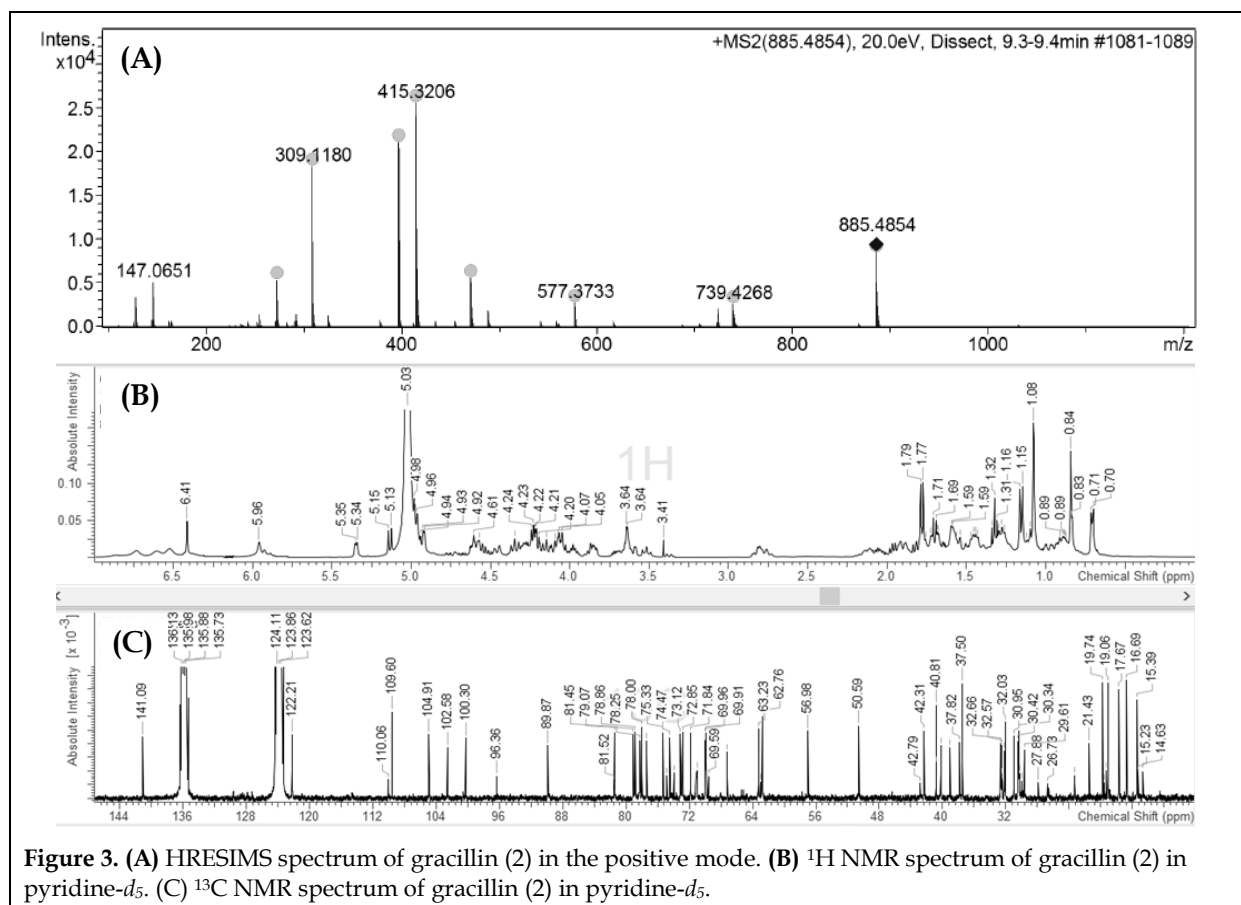
Compound **2** was obtained as a white amorphous powder. The HRESI-MS displayed a protonated ion peak [M+H]⁺ at *m/z* 885.4854 [M+H]⁺ in accordance with a molecular formula of C₄₅H₇₂O₁₇. Other fragments ions at *m/z* 723.4317 [M+H-162]⁺, 557.3738 [M+H-162-146]⁺, 415.3205 [M+H-162-146-162]⁺ and 397.3101 [M+H-180-146-162-18]⁺, presenting the respective elimination of a terminal hexose, a deoxyhexose, and a hexose moieties. Compound **2** showed to contain three sugar residues from the HMQC spectrum. The anomeric protons at δ_H 6.41 (br s), δ_H 5.14 (d, *J* = 7.83 Hz) and δ_H 4.97 (d, *J* = 7.46 Hz), giving correlations with carbon signals at δ_C 102.6, δ_C 104.9 and δ_C 100.3, were assigned as the anomeric protons of α-rhamnopyranose, β-glucopyranose, and β-glucopyranose, respectively (Fig. 3). Compound **2** showed to have the same MS/MS fragmentation pattern of gracillin and showed negative specific rotation [α]_D²⁵ = -46.9° (c 0.7, MeOH) as literature [α]_D¹⁴⁻²⁵ = (-83.2 - 88.6°, pyridine) (Du et al., 2002; Hernández et al., 2004). From these data, compound **2** was identified as gracillin (diosgenin 3-O-α-L-rhamnopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside) (Fig. 4).

After the characterization of the two saponins, they were tested against HL60 cell line. Dioscin (**1**) and gracillin (**2**) showed IC₅₀ values of 5.3 and 14.0

μM, respectively, and the positive control, etoposide showed an IC₅₀ value of 1.3 μM (Table 1). The isolated compounds (**1** and **2**) did not demonstrate selectivity comparison to HEK-293 cells since their SI values were 1.1 and 0.6, respectively. The IC₅₀

and SI values to etoposide in this cell line were not possible to be calculated once that at the highest concentration tested (100 μM), it did not reach 50% of inhibition of cell viability.





DISCUSSION

In our previous studies about bioactive natural compounds, we demonstrated the cytotoxic properties of the *C. subsessilis* against some cancer cell lines (Siqueira et al., 2016). Between all fractions tested, the CHCl₃ Fr showed the best cytotoxicity result against the HL60 cell line (IC₅₀ = 2.6 μg/mL

and SI = 7.2), and thus it was selected to isolate the active compounds.

There are some previous studies about *Costaceae* species that have demonstrated the cytotoxic activity of the *Costus pulverulentus* against PC3 cells (IC₅₀ value of 179 μg/mL) (Alonso-Castro et al., 2016), *Costus pictus* against HT1080 cells (IC₅₀ value of 120 μg/mL), HT-29 (IC₅₀ value of 125 μg/mL) and A549 cells (IC₅₀ values of 125 μg/mL) (Nadumane et al., 2011; Sathuvan et al., 2012) and, *Costus malortieanus* against Hep G2 cells (IC₅₀ value of 800 μg/mL) and WRL 68 cells (IC₅₀ value of 1260 μg/mL) (Al-Rashidi et al., 2011). However, these works do not state which compound is responsible for the cytotoxic activity of the plant.

Dioscin (1) and gracillin (2) were isolated as active compounds from REEX of *C. subsessilis*. They are spirostanol saponins with a β-D-glucopyranoside as the first sugar, α-L-rhamnopyranose substituted at the 2-position and another terminal sugar at the 4-position (dioscin) or 3- (gracillin)

Table 1. IC₅₀ values of the chloroform fraction and isolated compounds from *C. subsessilis* against cancer cell lines using MTT assay.

Sample	HL60 ^a	HEK-293 ^b	SI
	CI ₅₀ µg/mL (µM)		
CHCl ₃ Fr	15.1 ± 7.7*	6.0 ± 1.0	0.4
Dioscin (1)	4.6 ± 0.5 (5.3)	5.0 ± 2.9 (5.8)	1.1
Gracillin (2)	12.4 ± 5.0 (14.0)*	7.5 ± 3.1 (8.5)	0.6
Etoposide	0.8 ± 0.7 (1.3)	-	-

^aHuman promyelocytic leukemia; ^bHuman embryonic renal non-tumor. Samples were tested at eight nonserial dilutions from 100 to 0.6 µg/mL. All results were presented as mean ± SD, of three individual experiments. Triplicate samples were tested in each individual experiment (n = 9).
*Statistically significant compared with etoposide as control (p<0.05). No differences were observed among the samples against HEK-293.

(Zou et al., 2003). They were first isolated from *Dioscorea species* (Honda, 1904) and isolated for the first time in a plant of the family *Costaceae* (*Costus speciosus*) by Tschexhe and Pandey (1978).

In a previous work, gracillin showed cytotoxic activity (IC₅₀ value of 4.0 ± 0.4 µM) against HL-60 cell line (Hernández et al., 2004) and against the most eight solid tumor cell lines tested (with GI₅₀ < 100 µM), but it was not considered a potential anticancer candidate due to lack of selectivity against these cancer lines (Hu and Yao, 2003), corroborating with our results since we found a SI < 10 for compound 2. It is also known the cytotoxic (Chiang et al., 1991) and hemolytic (Takeshi et al., 1991) activities of dioscin. However, it was demonstrated that a 90-day subchronic toxicity study with dioscin in rats at a dose of 300 mg/kg/day (highest dose tested) caused gastrointestinal tract distension and hemolytic anemia only in male group comparison to the female group, providing evidence on the safety of this saponin for potential clinical application (Xu et al., 2012).

Based on a phylogenetic analysis of the family *Costaceae* (*Zingiberales*) Specht and Stevenson (2006) showed that *Costus* genus is found to be paraphyletic, with species occurring in the four clades: the *Cheilocostus* clade, the *Chamaecostus* clade, the *Paracostus* clade, and the *Costus* clade. The phylogenetic analyses of the South American *Costaceae* proposed *Chamaecostus* (four-taxon) as a

new genus (Specht, 2006). Thus, the chemical study of the *C. subsessilis* could help further chemotaxonomic classification studies to better understand the phylogenetic relationships of *Costaceae* family.

CONCLUSIONS

The chloroform fraction from the rhizomes ethanol extract of *C. subsessilis* showed promising cytotoxic activity corroborating the popular use of plant species of the *Costaceae* family for the treatment of tumors. The bio-guided chromatographic fractionation using MTT assay with HL60 cells of chloroform fraction from *C. subsessilis* furnished two saponins, dioscin and gracillin, responsible for its cytotoxic activity. To the best of our knowledge, this is the first time that these saponins are isolated from this species, and these findings could support the *Costaceae* family chemotaxonomic studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

- Alonso-Castro AJ, Zapata-Morales JR, González-Chávez MM, Carranza-Álvarez C, Hernández-Benavides DM, Hernández-Morales A (2016) Pharmacological effects and toxicity of *Costus pulverulentus* C. Presl (Costaceae). *J Ethnopharmacol* 180: 124–130.
- Al-Rashidi W, Supri NN, Manshoor N (2011) Cytotoxic activity of the crude extract from *Costus malortianus* (COSTACEAE). *Am-Eurasian J Toxicol Sci* 3(2): 63–66.
- André T, Specht C, Salzman S, Palma-Silva C, Wendt T (2015) Evolution of species diversity in the genus *Chamaecostus* (Costaceae): molecular phylogenetics and morphometric approaches. *Phytotaxa* 204(4): 265.
- Chiang HC, Tseng TH, Wang CJ, Chen CF, Kan WS (1991) Experimental antitumor agents from *Solanum indicum* L. *Anticancer Res* 11(5): 1911–1917.
- Du SH, Liu WY, Fu TJ, Xia BG, Xia CD (2002) Isolation and identification of steroidal saponins in total saponin from *Dioscorea nipponica* Makino. *Yao Xue Xue Bao* 37(4): 267–270.
- Hernández JC, Leon F, Quintana J, Estevez F, Bermejo J (2004) Icogenin, a new cytotoxic steroidal saponin isolated from *Dracaena draco*. *Bioorg Med Chem* 12(16): 4423–4429.
- Honda J (1904) Untersuchungen über die saponinsubstanzen der *Dioscorea* Tokoro Makino. *Arch Exp Pathol Pharmacol* 51(2-3): 211–226.
- Hu K, Yao X (2003) The cytotoxicity of methyl protoneogracillin (NSC-698793) and gracillin (NSC-698787), two steroidal saponins from the rhizomes of *Dioscorea collettii* var. *hypoglauca*, against human cancer cells *in vitro*. *Phytother Res* 17(6): 620–626.
- Jha MK, Alam MB, Hossain MS, Islam A (2010) *In vitro* antioxidant and cytotoxic potential of *Costus speciosus* (Koen.) smith rhizome. *Int J Pharm Sci* 1(10): 138–144.
- Mahato SB, Sahu NP, Ganguly AN (1980) Carbon-¹³ NMR spectra of dioscin and gracillin isolated from *Costus speciosus*. *Indian J Chem B* 19B (9): 817–819.
- Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 65 (1-2):55–63.
- Nadumane VK, Rajashekar S, Narayana P et al. (2011) Evaluation of the anticancer potential of *Costus pictus* on fibrosarcoma (HT-1080) cell line. *J Nat Pharm* 2(2):72–76.
- Newman DJ, Cragg GM (2016) Natural products as sources of new drugs from 1981 to 2014. *J Nat Prod* 79(3): 629–661.
- Pettit GR, Zhang Q, Pinilla V, Hoffmann H, Knight JC, Doubek DL, Chapuis JC, Pettit RK, Schmidt JM (2005) Antineoplastic agents. 534. Isolation and structure of sansevistatins 1 and 2 from the African *Sansevieria ehrenbergii*. *J Nat Prod* 68(5): 729–733.
- Pilla MAC, Amorozo MCM, Furlan A (2006) Obtenção e uso das plantas medicinais no distrito de Martim Francisco, Município de Mogi-Mirim, SP, Brasil. *Acta Bot Bras* 20(4): 789–802.
- Sathuvan M, Vignesh A, Thangam R, Palani P, Rengasamy R, Murugesan K (2012) *In vitro* antioxidant and anticancer potential of bark of *Costus pictus* D. DON. *Asian Pac J Trop Biomed* 2(2): S741–S749.
- Siqueira EP, Ramos JP, Zani CL, Nogueira ACO, Nelson DL, de Souza-Fagundes EM, Cota BB (2016) *Chamaecostus subsessilis* and *Chamaecostus cuspidatus* (Nees & Mart) C. Specht and D.W. Stev as potential sources of anticancer agents. *Nat Prod Chem Res* 4(2): 1000204.
- Specht CD, Stevenson DW (2006) A new phylogeny-based generic classification of Costaceae (Zingiberales). *Taxon* 55 (1): 153–163.
- Spech CD (2006) Systematics and evolution of the tropical monocot family Costaceae (Zingiberales): A multiple dataset approach. *Syst Bot* 31(1): 89–106.
- Takechi M, Shimada S, Tanaka Y (1991) Structure-activity relationships of the saponins dioscin and dioscinin. *Phytochemistry* 30(12): 3943–3944.
- Tschexhe R, Pandey VB (1978) Steroidal saponins of *Costus speciosus*. *Phytochemistry* 17(10): 1781–1782.
- Xu T, Zhang S, Zheng L, Yin L, Xu L, Peng J (2012) A 90-day subchronic toxicological assessment of dioscin, a natural steroid saponin, in Sprague-Dawley rats. *Food Chem Toxicol* 50(5): 1279–1287.
- Zou CC, Hou SJ, Shi Y, Lei PS, Liang XT (2003) The synthesis of gracillin and dioscin: two typical representatives of spirostanol glycosides. *Carbohydr Res* 338(8): 721–727.
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AUTHOR CONTRIBUTION:

Contribution	Siqueira EP	Galligani F	Braga ACS	Souza-Fagundes EM	Cota BB
Concepts or ideas					x
Design				x	x
Definition of intellectual content				x	x
Literature search					x
Experimental studies	x	x	x	x	x
Data acquisition	x	x	x		x
Data analysis	x	x	x		x
Statistical analysis				x	
Manuscript preparation	x				x
Manuscript editing					x
Manuscript review	x	x	x	x	x

Citation Format: Siqueira EP, Galligani F, Braga ACS, Souza-Fagundes EM, Cota BB (2020) Saponins from the rhizomes of *Chamaecostus subsessilis* and their cytotoxic activity against HL60 human promyelocytic leukemia cells. J Pharm Pharmacogn Res 8(5): 466–474.