Research Article

Isaac Silvère Gade*, Corinne Chadeneau, Richard Tagne Simo, Emmanuel Talla, Alex De Theodore Atchade, Paule Seité, Brigitte Vannier, Sophie Laurent, Celine Henoumont, Armel H. Nwabo Kamdje, Jean-Marc Muller

A new phenyl alkyl ester and a new combretin triterpene derivative from Combretum fragrans F. Hoffm (Combretaceae) and antiproliferative activity

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Abstract: This work concerns the isolation and structure elucidation of compounds obtained from the extract of leaves and stem bark of Combretum fragrans F. Hoffm. Both extracts and some isolated compounds were tested for antiproliferative activity on glioblastoma (U87MG and C6 cells) and prostate (PC-3 cells) cancer cell lines using XTT (2,3-bis[2-methoxy-4-nitro-5-sulphonyl]-2H-tetrazolium-5-carboxanilide inner salt) assay. The dichloromethane/methanol (1:1) extract of the leaves led to the isolation of two new compounds such as fragransinate (1) and combretin C (2), alongside five known compounds such as combretin A (3), belamcanidin (4), cirsilineol (5), velutin (6), and a mixture of β-sitosterol-3-O-β-D-glucopyranoside (10a) and stigmasterol-3-O-β-D-glucopyranoside (10b), whereas the methanol extract of the stem bark led to the isolation of three known compounds betulnic acid (7), bellericagenin B (8), and a mixture of β-sitosterol (9a) and stigmasterol (9b). The structure of compounds was elucidated by nuclear magnetic resonance and mass spectrometry data. The methanol extract of the stem bark showed a powerful antiproliferative activity on all tested cells, as well as the extract of leaves which also showed important cytotoxicity effect. Compound (3) showed good antiproliferative activity particularly on U87MG and PC-3 cells, whereas compound (5) exhibits moderated activity. Compounds (2) and (8) were not active on all tested cells.

Keywords: Combretum fragrans F. Hoffm, fragransinate, combretin C, antiproliferative activity

1 Introduction

The genus Combretum is the largest and most widespread genus of Combretaceae. It is widely distributed in tropical and subtropical regions (Africa, India, and China) and comprises about 200 and 250 species that include trees, shrubs, shrublets, or woody climbers [1]. The genus Combretum is reported to have various biological activities such as antioxidant, anti-inflammatory, antimalarial, antibacterial, and cytotoxicity against tumor cells [1,2]. Phytochemical studies of Combretum genus showed many secondary metabolites such as flavonoids, triterpenes, ligans, phenanthenes, and stibenoids. [1,2]. Combretum fragrans F. Hoffm (syn: Combretum adenogenium Steud. ex A. Rich) is a medicinal plant belonging to the Combretum genus. It is a shrub or a small tree that grows up to 10–12 m high. The plant is found in deciduous woodland, wooded grassland associated with seasonally waterlogged clay soils, and on shallow, stony soils [3]. C. fragrans F. Hoffm is used in African folk medicine for the treatment of various types of disease, such as coughs, syphilis, leprosy, septic wounds, fungal infection of the scalp, diarrhea, hypertension, incurable wound, malaria, snake bite, gonorrhea, pain, and inflammation [4–6]. In the northern part of Cameroon, the plant is used to treat jaundice, diabetic wound, ulcers, and cancers.
Pharmacological investigation of *C. fragrans* F. Hoffm extracts have been established to have antibacterial, antifungal, and antiproliferative properties [7,8], whereas phytochemical analysis of leaves, stem bark, and roots has revealed the presence of flavonoids, triterpenes, sterols, saponins, and tannins [9,10]. Despite the well-documented benefits of *C. fragrans* F. Hoffm, only few phytochemical studies have been performed on it.

The search of novel bioactive compounds against cancer is still on, because many cancer patients develop resistance to the existing anticancer agents during treatment and because of the undesired side effects produced by these anticancer agents. Plants offer a large possibility to find novel anticancer agents with new mechanism of action and weak toxicological effect. It is in this sense that we investigated *C. fragrans* F. Hoffm. The present work describes the isolation of compounds from extract of leaves and stem bark of *C. fragrans* F. Hoffm and evaluation of antiproliferative activity of both extracts and some isolated compounds on glioblastoma (GBM) and prostate cancer cell lines.

2 Materials and methods

2.1 General experimental procedure

The melting point of the new compounds was recorded in open capillary using Electrothermal 9100 and is uncorrected. IR spectra were recorded on FT-IR spectrometer (Perkin-Elmer Spectrum 100). The proton and 13C carbon nuclear magnetic resonance (NMR) data were recorded on spectrometer Bruker Avance AV-500 and 600, and tetramethylsilane (TMS) was used as standard. Chemical shifts are given in ppm (δ) and coupling constant (J) in Hz. CDCl₃, MeOD, and DMSO-d₆ were used as NMR solvents. Electrospray ionization-mass spectrometry (ESI-MS) spectra were registered on a QTOF Spectrometer (Bruker, Germany) equipped with a ZQ FI source. The spectrometer was operated in positive mode (scan: 150–1,500; centroid CV = 20). The high resolution mass spectrum was registered on LC-MS-QTOF Spectrometer (Bruker, Germany) equipped with ESI source operating in positive mode. Column chromatography (CC) was performed on silica gel 60 (70–230 mesh, Merck), and thin layer chromatography was performed on silica gel precoated plates F-254 Merk (20 × 20 cm). Spots were visualized under UV light (254 and 365 nm), sprayed with 5% of phosphomolybdic acid prepared in ethanol, then heated. XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt) reagent from Promega was used to evaluate antiproliferative activity.

2.2 Plant material

Leaves and stem bark of *C. fragrans* F. Hoffm were collected during the month of May 2017 at Padarmé located in Bibémi subdivision, in the north region of Cameroon. The sample was identified at the National Herbarium of Cameroon, where a voucher specimen is deposited under the number 39,753/HNC.

2.3 Extraction and isolation

The powders of 1 kg of leaves and 1 kg of stem bark of *C. fragrans* F. Hoffm were extracted separately by maceration at room temperature with 5 L of dichloromethane/methanol (CH₂Cl₂/MeOH) (1:1) and methanol (MeOH) respectively for 48 h with intermittent stirring. After filtration on Whatman filter paper, the resulting solutions were concentrated using a rotary evaporator. The procedure was repeated thrice to optimize extraction. We obtained 55 g of crude extract from the leaves and 40 g from the stem bark. Then, a quantity of 30 g of each crude extract was taken differently and each subjected to CC separation on silica gel with the gradient eluting system hexane–ethyl acetate (100:0 → 0:100), followed by ethyl acetate–methanol (100:0 → 0:100). The fractions of 100 mL each were collected and concentrated on rotary evaporator. From the extract of leaves, two new compounds, fragransinate (1, 5.1 mg) and combretin C (2, 215 mg), alongside four known compounds combretin A (3, 46 mg), belamcanidin (4, 12.3 mg), cirsilineol (5, 64 mg), and velutin (6, 60 mg), and a mixture of β-sitosterol-3-O-β-D-glucopyranoside and stigmasterol-3-O-β-D-glucopyranoside (10a,b, 9 mg) were isolated. From the methanol crude extract of the stem bark, three known compounds: betulinic acid (7, 4.3 mg), bellericagenin B (8, 13 mg), and a mixture of β-sitosterol and stigmasterol (9a, b, 15.6 mg) were isolated (Figure 1).

2.3.1 Compound 1: fragransinate (3′-3′,4′-dihydroxy-5-methoxyphenyl)propyl heptadecanoate

Whitish solid; C₂₂H₄₆O₅; m.p. = 66.5–67.5; IR: νmax = 3,400 cm⁻¹ (−OH), 1,735 cm⁻¹ (C=O), 1,450–1,600 cm⁻¹
2.3.2 Compounds 2: combretin C (3-β-D-xylopyranosyl)-15α-hydroxy-cycloarten-28-oic acid

White powder; C_{x} H_{y} O_{z}; m.p. = 239.1–240.1; IR: \nu_{\text{max}} = 3,400 \text{ cm}^{-1} (-\text{OH}), 1,700 \text{ cm}^{-1} (\text{C} = \text{O}) and 1,000–1,025 \text{ cm}^{-1} (\text{C} = \text{O}); High resolution ESI mass spectrum (HRESI-MS) (+) HRESI-MS m/z 643.4149 [M + Na]^+ (calculated for C_{x} H_{y} O_{z}, 643.4288); 1H and 13C NMR data, see Table 2.

2.4 Biological evaluation

2.4.1 Cell culture and cell conditions

The U87 human GBM cells line and C6 rat GBM cells line were maintained in Dulbecco’s modified Eagle’s medium.
**Table 1:** $^1$H and $^{13}$C NMR data for compounds 1 (CDCl$_3$, $\delta$ in ppm, 600 and 151 MHz)

| No. | $\delta_H$ (Mult., $J$/Hz) | $\delta_C$ |
|-----|----------------|-----------|
| 1   | —               | 174.0     |
| 2   | 2.33 (t, $J = 7.5$; 2H) | 34.4      |
| 3   | 1.64 (dd, $J = 7.5, 14.2$; 2H) | 25.0      |
| 4–16| 1.73–2.12 (m) | 29.2–29.7 |
| 17  | 0.90 (t, $J = 6.9$; 3H) | 14.1      |
| 1'  | 4.10 (t, $J = 6.5$; 2H) | 63.5      |
| 2'  | 1.93 (dd, $J = 7.6, 6.5$; 2H) | 32.1      |
| 3'  | 2.60 (t, $J = 7.6$ Hz; 2H) | 30.3      |
| 1'' | —               | 133.1     |
| 2'' | 6.30 (s, 1H) | 103.2     |
| 3'' | —               | 146.7     |
| 4'' | —               | 130.5     |
| 5'' | —               | 143.7     |
| 6'' | 6.45 (s, 1H) | 108.3     |
| MeO− | 3.89 (s, 3H) | 56.11     |

(DMEM, 1 g/L glucose) with GlutaMAX™ (Gibco) and sodium pyruvate (Invitrogen), supplemented with 10% of fetal calf serum and 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco) (antibiotics mixture). The PC-3 human prostate cancer cells line were maintained in the same condition except the use of DMEM medium with 4.5 g/L glucose. Cell cultures were carried out in a biological safety cabinet (laminar flow hood). An inverted microscope (OCO-2-KERN) was used for cell counting. All cells line were grown in a humidified incubator (95% air and 5% CO$_2$) at 37°C.

**2.4.2 Antiproliferative assay**

The antiproliferative activity of extracts and some isolated compounds was evaluated using XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt) test [11]. The assay is based on the reduction of XTT reagent (Promega) into an orange formazan dye by the metabolically active living cells. Thus, the U87 and C6 GBM cells, as well as the PC-3 prostate cancer cells were plated in 96-well plates respectively at a density of $5 \times 10^3$, $3 \times 10^3$, and $4 \times 10^3$ cells per well in 100 µL of the corresponding medium. Then, cells were allowed to attach overnight in growth medium. After 24 h of incubation, the medium was removed and replaced with fresh medium containing different concentrations (3.15–150 µg/mL) of extracts or compounds, and control cells were treated with DMSO (0.5%) used to dissolve samples. After 72 h of treatment, 50 µL of XTT labeling reagent mixture solution was added in each well and the plate was incubated for 4 h. After incubation with the XTT reagent, the formazan formed is soluble in the medium and is directly proportional to the living cells. Then, cell viability was assessed by the measurement of the absorbance at 490 nm using a spectrophotometric microplate reader.

Each test was performed in triplicate. The IC$_{50}$ (50% inhibitory concentration, that is, the concentration of extract or compound that inhibits 50% of the proliferation of cancer cells) value was calculated automatically from the sigmoidal dose–response nonlinear regression curve using GraphPad prism software.

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**Table 2:** $^1$H and $^{13}$C NMR data for compounds 2 (DMSO-$d_6$, $\delta$ in ppm, 500 and 126 MHz)

| No. | $\delta_H$ (Mult., $J$/Hz) | $\delta_C$ |
|-----|----------------|-----------|
| 1   | 1.24$^a$, 1.08$^a$ | 32.3      |
| 2   | 1.81$^a$, 1.52$^a$ | 28.5      |
| 3   | 3.96 (dd, $J = 11.0, 4.7$; 1H) | 82.4      |
| 4   | —               | 58.8      |
| 5   | 1.92$^a$ | 44.7      |
| 6   | 1.58$^a$, 1.15$^a$ | 24.6      |
| 7   | —               | 27.6      |
| 8   | —               | 46.9      |
| 9   | —               | 19.3      |
| 10  | —               | 26.0      |
| 11  | —               | 27.1      |
| 12  | —               | 34.7      |
| 13  | —               | 48.3      |
| 14  | —               | 44.6      |
| 15  | 3.24 (m, 1H) | 71.2      |
| 16  | 1.85$^a$ | 43.9      |
| 17  | 1.57$^a$ | 51.5      |
| 18  | 1.01 (s, 3H) | 18.9      |
| 19  | 0.35 (d, $J = 4.0$; H-19b), 0.54 (d, $J = 4.0$; H-19a) | 28.8      |
| 20  | 1.32$^a$ | 38.9      |
| 21  | 0.81 (d, 3H) | 18.6      |
| 22  | 1.71$^a$, 1.24$^a$ | 34.9      |
| 23  | —               | 30.8      |
| 24  | —               | 35.1      |
| 25  | 2.20 | 36.2      |
| 26  | 0.86 (brd, 3H) | 25.7      |
| 27  | 0.86 (brd, 3H) | 25.0      |
| 28  | —               | 177.9     |
| 29  | 1.03 (s, 3H) | 10.0      |
| 30  | 0.95 (s, 3H) | 14.3      |
| 31  | 1.00 (brd, 3H) | 18.6      |

**Xylose**

| No. | $\delta_H$ (Mult., $J$/Hz) | $\delta_C$ |
|-----|----------------|-----------|
| 1   | 4.14 (d, $J = 7.5$; 1H) | 104.0     |
| 2   | 2.89 (brdd, 1H) | 73.4      |
| 3   | 3.02 (brdd, 1H) | 76.3      |
| 4   | 3.23 (brdd, 1H) | 69.4      |
| 5   | 3.65 (brdd, Ha-5'); 2.97 (brdd, Hb-5') | 65.5      |

$^a$ Signals overlapped.
Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Structural elucidation of compounds

Chromatographic separation on silica gel of the CH₂Cl₂/MeOH (1:1) crude extract of leaves of *Combretum fragrans* F. Hoffm led to two new compounds (1 and 2) and five known compounds (3, 4, 5, 6, and 10), whereas the methanol crude extract from the stem bark yielded three known compounds (7, 8, and 9). The structures of the compounds were elucidated by spectral analysis using ESI-MS and 1D, 2D NMR data.

Compound 1 was isolated as a whitish powder and melted between 66.5 and 67.5°C. Its molecular formula, 

\[ C_{27}H_{46}O_5 \text{, implying MS and 1D, 2D NMR data.} \]

pounds were elucidated by spectral analysis using ESI

with aliphatic methylene

of a terminal methyl signal at

1H NMR analysis revealed in up

3.1 Structural elucidation of compounds

all signals of aromatic protons

The two signals at δc 143.7 and 146.7 indicate that two of the oxygenated quaternary carbons are in *ortho* position. The \( ^{1}H-^{13}C \) COSY experiment shows correlations between the oxymethylene proton at δH 4.10 and the methylene proton at δH 1.93, which in turn correlates with another methylene proton at δH 2.59. These correlations suggest a propene chain substituted on position C-1′ and C-3′ enabling the assignment of chemical shifts at 4.10 to H-1′, 1.93 to H-2′, and 2.59 to H-3′. The spectrum also shows correlations between the \( \alpha \) and \( \beta \) protons to a carboxyl (ester) respectively at 2.31 (H-2) and 1.63 (H-3). The proton in \( \beta \) correlates in turn with the proton at δH 1.33 (H-4). The heteronuclear multiple bond correlation (HMBC) experiment shows correlations between the proton at δH 4.10 (H-1′) and carbons at δc 32.0 (C-3′), 30.5 (C-2′) and a carbonyl signal at 173.9 (C-1). The spectrum also displays correlation between the proton at δH 1.93 (H-2′) and carbons at δc 31.6 (C-3′), 63.3 (C-1′) as well as with an aromatic carbon signal at 133.2 (C-1″). Cross-peak correlations were also observed between the proton at δH 2.59 (H-3′) and aromatic carbons at 108.3 (C-6″), 103.1 (C-2″), and 133.2 (C-1″) as well as carbons at δc 30.2 (C-2′), 33.4 (C-1′). Proton H-3 is the only proton that has several correlations with carbons from phenyl ring, indicating that C-3 is directly attached to the phenyl ring on C-1″ position. This result suggests that the aliphatic chain, which is a fatty ester, is located on C-1′ position of the propyl chain. In addition, the HMBC spectrum shows correlations of an aromatic proton at δH 6.45 (C-2″) with carbons at δc 31.9 (C-3′), 108.6 (C-6″), 130.2 (C-4″), and 143 (C-3″). The aromatic proton at δH 6.30 (H-6″) correlates with different carbons at δc 31.9 (C-3′), 103.2 (C-2″), 130.3 (C-4″), and 146.7 (C-5″). Proton of the methoxy group (δH 3.89) correlates with the carbon at δc 146.7, indicating that the methoxy group is attached on C-5″ position of the aromatic ring. Important HMBC correlations were also observed between the proton at δH 2.31 (H-2) from the long chain and carbons at δc 29.02 (C-4), 25.05 (C-3), and the carbonyl function at δc 173.98 (C-1). The length of the aliphatic chain was determined in conjunction with its \( ^{1}H \) NMR spectrum and different fragmentation patterns observed on its mass spectrum. Characteristic fragments appeared at \( m/z \) 311, 253, and 181 (Figure S1). All these spectral data and some information
reported in literature enabled us to characterize compound 1 as 3’-(3’4’-di-hydroxy-5-methoxyphenyl)propyl heptadecanoate to which the trivial name fragransinate has been given. To the best of our knowledge, this compound is isolated for the first time from natural sources.

Compound 2 was isolated as a white powder and melted between 239.1 and 240.1°C. Its HRESI-MS mass spectrum in positive mode shows the pseudomolecular ion peak at m/z 643.4149 [M + Na]⁺ (calculated for C₃₆H₆₀O₈, 643.4288) corresponding to the molecular formula C₃₆H₆₀O₈ accounting seven degrees of unsaturation. The IR spectrum exhibits absorption bands ν max at 3,400 cm⁻¹, 1,700 cm⁻¹, and 1,000–1,025 cm⁻¹, which were respectively attributable to a hydroxyl group, a carbonyl group, and C–O bonds. The analysis of its ¹H NMR spectrum in conjunction with HSQC spectrum shows in upfield region the signals of two protons as doublet at δH 0.35 (1H, d, j = 4.0 Hz) and δH 0.54 (1H, d, j = 4.0 Hz) corresponding respectively to proton H-19b and H-19a of a cyclopropane from a cyclooctane-type triterpene [13]. The spectrum also revealed the presence of six methyl groups at δH 0.80, 0.86, 0.92, 1.00, 1.01, and 1.03, and two oxymethine proton signals appearing at δH 3.26 (1H, m) and 3.96 (1H, dd, j = 11.0; 4.7 Hz). The spectrum also displays signals of osidic proton indicating the presence of a sugar moiety. Signals of oxymethine protons of a sugar moiety appeared at δH 3.67 (1H, dd, j = 11.3; 5.3 Hz), 3.24 (1H, m), 3.02; 2.97 (1H, m), 2.89 (1H, m) as well as the anomic proton at 4.14 (1H, d, j = 7.5 Hz). The larger coupling constant (j = 7.5 Hz) of the anomic proton suggests the β-configuration of the sugar moiety. The ¹H NMR spectrum of compound 2 was very similar to that of Combretin B [9], except the missing signal of an olefinic proton in 2. Combretin B has been previously isolated from the same plant. The sugar moiety was identified as xylose. The ¹³C NMR spectrum confirmed the absence of olefinic function compared with that of Combretin B. The spectrum shows signals of 36 carbons including three methyl group signals as singlet at δC 18.9 (C-18), 10.0 (C-29), and 14.3 (C-30); four methyl group signals as doublet at δC 18.6 (C-21), 25.7 (C-26), 25.0 (C-27), and 18.6 (C-31); two oxymethine signals at δC 82.4 (C-3) and 71.2 (C-15); one carbonyl function at δC 177.5 (C-28). The signals of sugar moiety appear at δC 73.4 (C-2’), 76.3 (C-3’), 69.4 (C-4’), and 65.5 (C-5’) together with the anomic carbon at δC 104.0 attributable to C-1’. The HMBC experiment shows important correlation between the anomic proton at δH 4.14 and the carbon at δC 82.43 (C-3), indicating that the sugar moiety is attached to the aglycone moiety at the C-3 position. Other correlations were also observed between the proton at δH 1.03 (H-29) and carbons at δC 177.59 (C-28), 82.43 (C-3), and 53.54 (C-4), indicating that the carbonyl group is located on C-28 position. According to the great similarity observed between compound 2 and Combretin B, the structure of compound 2 has been proposed as 3-(β-D-xlyopyranosyl)-15α-hydroxyoctan-28-oic acid for which the trivial name Combretin C has been given. The compound is isolated for the first time as a new derivative combretin triterpene.

The known compounds were identified from their NMR spectroscopic data and comparisons made with those reported in the literature: combretin A (3) [9]; belamcanidin (4), cirsilineol (5), velutin (6) [14]; betulinic acid (7) [15], bellericagenin B (8) [16], a mixture of β-sitosterol (9a) and stigmasterol (9b) [17]; and a mixture of β-sitosterol-3-O-β-D-glucopyranoside (10a) and stigmasterol-3-O-β-D-glucopyranoside (10b) [18]. Compounds 7, 8, 10a, and 10b are reported for the first time from this plant species.

3.2 Antiproliferative activity

The antiproliferative activity of extracts and compounds 2, 3, 5, and 8 of C. fragrans F. Hoffm on U87 human GBM cells, C6 rat GBM cells, and PC-3 prostate cancer was evaluated by XTT test. From the results presented in Table 3, it is deduced that the MeOH crude extract of the stem bark of C. fragrans F. Hoffm was found to be more active on all tested cells than the CH₃Cl/MeOH (1:1) crude extract from the leaves. The MeOH extract powerfully inhibits the proliferation of U87, C6, and PC-3 cells after 72 h treatment compared to untreated control cells. The extract was most active on PC-3 prostate cancer cells (IC₅₀ = 11.24 µg/mL) than C6 (IC₅₀ = 12.17 µg/mL) and U87 (IC₅₀ = 20.13 µg/mL) cancer cells. On the contrary, the CH₃Cl/MeOH extract from the leaves also showed important inhibition of C6 cells proliferation (IC₅₀ = 14.19 µg/mL) and moderate cytotoxicity effect on U87 (IC₅₀ = 31 µg/mL) and PC-3 (36.26 µg/mL) cells. The literature mentions that many species from the combretum genus, similar to the specie studied here, are known to possess antiproliferative activity [8].

Among the tested compounds, combretin A (3) was the most active followed by compound cirsilineol (5), whereas combretin C (2) and bellericagenin B (8) were not active. Compound 3 exhibited very good inhibition on PC-3 cells proliferation (IC₅₀ = 22.57 µM), as well as on U87 (IC₅₀ = 29.30 µM) and C6 cells (58.93 µM) which is moderated. Compound 5 also showed important
inhibition on PC-3 (IC$_{50}$ = 30.46 µM) and C$_6$ (IC$_{50}$ = 31.61 µM) cells’ proliferation and weak effect on U87 cells growth (IC$_{50}$ = 65.46 µM). Compounds 3 and 5 isolated from the leaves could be responsible for the antiproliferative activity obtained with the related extract.

Combretin A (3) and C (2) are cycloartane-type triterpenes. The second is different from the first just by the presence in its structure of a sugar moiety on carbon C-3 and by the absence of a double bond on C-24 and C-31 carbons. However, combretin A showed good activity on all tested cells, whereas combretin C was inactive. The loss of the activity in combretin C could be attributable to the presence in its structure of the sugar moiety and the lack of the double bonds which negatively influences its activity. Previous study concerning structure–activity relationship showed that sugar moiety in gensenoside decreases its anticancer potential, whereas its aglycone presents good anticancer activity [19]. This study could explain our obtained results. Cycloartane triterpene types are also known for their anticancer activity [20,21], and this corroborates with the result obtained with combretin A. This is the first time that Combretin A is evaluated for its antiproliferative activity and it could be a good candidate for developing an anticancer agent in a pharmaceutical area.

Compound (5) or cirsinelineol is a flavonoid from flavonone type. Its activity was moderated on C6 and PC-3 cells but weak on U87MG cells. Its antiproliferative activity has already been documented for the first time on a large panel of cancer cell lines [22], and the results are similar with those obtained here with PC-3 cells. Flavonoids are known to possess anticancer activity, and this activity correlates positively with the presence of methoxyl group, carbonyl on carbon C-4, and the double bond on carbon C-2 and C-3 of flavonoids [23].

Compound (8) or bellericagenin B is an oleanane-12-ene triterpene type polyhydroxylated and showed no activity on all tested cancer cells. It is known that hydroxyl group correlates negatively with the anticancer activity in flavonoids [23] and other compounds [24]. Thus, the number of hydroxyl groups in compound (8) could explain its inactivity. In addition, compound (8) could be an oleanolic acid derivative and is different from this later with four hydroxyl groups. However, it is known that oleanolic acid, which possesses one hydroxyl group, has very good anticancer activity [25,26].

Betulinic acid (7) and stigmasterol (9b) were isolated from the bark and are known for their anticancer activity on many types of cancers [27,28]. Thus, the higher antiproliferative activity observed with the MeOH crude extract of the stem bark could be attributable in part to the presence of compounds 7 and 9b. The results of the antiproliferative activity of both extracts and some compounds of C. fragrans support with scientific evidences the traditional use of this plant in the treatment of cancer.

### 4 Conclusion

The present work describes the isolation and antiproliferative activity of extracts and some isolated compounds from C. fragrans F. Hoffm on GBM and prostate cancer cells. Column chromatographic separation on silica gel of the CH$_2$Cl$_2$/MeOH crude extract from the leaves yielded two new compounds (1 and 2) and five known compounds (3, 4, 5, 6, and 10), whereas the MeOH crude extract from the stem bark led to three known compounds (7, 8, and 9). Compounds 7, 8, and 10 were detected for the first time from this species. The results of the antiproliferative activity against U87, C$_6$, and PC-3 cancer cells showed that the methanol crude extract from the stem bark exhibits powerful antiproliferative effect on all tested cells than the CH$_2$Cl$_2$/MeOH extract from leaves. As well, compound 3 was found to be the most active tested compound with highest effect on PC-3 cancer cells. The antiproliferative activity of extracts and compounds from C. fragrans F. Hoffm against U87, C$_6$, and PC-3 cancer cells is reported here for the first time. In the future, we plan to isolate compound 1 with the aim of obtaining more compounds and testing its antiproliferative activity. This work can also serve as a guide for further isolation of compounds from other parts of C. fragrans F. Hoffm and evaluation of their anticancer properties as well as their toxicity.

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### Table 3: Inhibitory concentration values of extracts and some compounds that kill or inhibit 50% of cancer cells (IC$_{50}$ in µg/mL for extracts, and µM for compounds)

| Extracts and compounds | U87 MG | C6 | PC-3 |
|------------------------|--------|----|------|
| CH$_2$Cl$_2$/MeOH extract | 31 µg/mL | 14.19 µg/mL | 36.26 µg/mL |
| MeOH extract | 20.13 µg/mL | 12.17 µg/mL | 11.24 µg/mL |
| 2 | >100 µM | >100 µM | >100 µM |
| 3 | 29.30 µM | 58.93 µM | 22.57 µM |
| 5 | 65.46 µM | 31.61 µM | 30.46 µM |
| 8 | >100 µM | >100 µM | >100 µM |
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