Chemical Analysis and in Vitro Antiproliferative Potential of *Eugenia uniflora* L. (Myrtaceae)

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Authors’ contributions

All authors contributed substantially to the work reported. Authors DBG and WARJ conceived and designed the experiments analyzed the data and wrote the paper. Authors BZ, PZS, DM, AMS, MECZ, AJP, CSC, GL and MFCS performed the experiments. Authors ALTGR, AB, and JL performed the experiments and contributed with materials, analysis tools and wrote the paper. All authors read and approved the final manuscript.

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ABSTRACT

Natural products and especially medicinal plants, have been extensively studied and have exhibited antiproliferative effects. The species *Eugenia uniflora* L. (Myrtaceae) is native to Brazil and distributed throughout Australia, East Asia, and the Americas. The leaves are commonly used for the treatment of diarrhea, fever, and hypertension. However, the chemical properties and antiproliferative potential of the extracts remain to be elucidated. In this work, the antiproliferative effects of hydroethanolic (HEE) and dichloromethane (DEE) extracts of leaves from *E. uniflora*.
against a panel of human tumor cell lines in vitro were evaluated. Quercetin was identified using an HPLC, and the chemical component β-sitosterol was isolated. The HEE was an inactive extract, whereas the DEE extract showed antiproliferative activity against OVCAR-3 ovarian cells. In a bioassay-guided process, more pronounced antiproliferative effects were found for β-sitosterol isolated from DEE, which demonstrated potent antiproliferative effects against glioblastoma (U-251 cells) and leukemia (K-562 cells). This study contributes to the knowledge of E. uniflora and suggests that β-sitosterol is a potential chemotherapeutic compound.

Keywords: Antitumor agents; β-sitosterol; medicinal plants; phytochemistry.

1. INTRODUCTION

Myrtaceae, one of the main Angiosperm families and is concentrated in a single tribe (Myrtaceae) and three subtribes (Myrcinae, Eugeniinae, and Myrtinae) [1,2]. Myrtaceae has a broad diversity of plants, distributed in South America, Australia, and Tropical Asia, with approximately 142 genera and 5,760 species [3,4]. In Brazil, the family includes 23 genera and approximately 1,000 species [5], which are used to produce paper (Eucalyptus spp.), food or juice (Psidium guajava L.), or as medicines, commonly infusions (Myrcia uniflora DC.) [6].

The species Eugenia uniflora L. is distributed throughout several countries, characteristically in tropical and subtropical regions [7]. Known as the Brazilian cherry tree (or “pitangueira”), it is a fruity tree found throughout the country [8], especially between the states of Minas Gerais and Rio Grande do Sul [9]. E. uniflora was introduced as an empirical medicine by Guarani Indians in the 15th century [10]; today, the leaves are often used for the treatment of inflammation, fever, hypertension, and diarrhea [11,7], as a diuretic, and to lower blood glucose levels [12,13].

Previous studies demonstrated that aqueous extract of E. uniflora leaves reduced blood pressure in rats through α-adrenergic antagonism as a direct vasodilator [14] and owing to diuretics effects [15]. Amorim et al. [16] verified the antinociceptive activity in mice by using essential oils obtained from of leaves of the plant, and Rattmann et al. [17] showed that the flavonoid-rich fraction obtained from fresh leaves reduced the lethality of cecal ligation and puncture (CLP) in mice through decreases in inflammatory mediators. These pharmacological effects are usually attributed to the presence in the leaves of E. uniflora, volatile terpenoid oils, condensed and hydrolysable tannins, flavonoids, leucoanthocyanidins, and steroids and/or triterpenoids [16]. These secondary metabolites have also shown potential as anticancer molecules [18].

Cancer, a disease characterized by the uncontrolled multiplication of modified normal cells, is a leading cause of death worldwide and represents a major public health burden [19,20]. The treatment of cancer represents a challenge as there is no single effective treatment that works for all types of cancer [21]. The treatment consists of chemotherapy, radiotherapy, surgery, and immunotherapy, or their combination. Chemotherapy, which employs different combinations of cytotoxic drugs, is often associated with serious adverse effects and chemoresistance [22]. Also, many cancers exhibit only modest clinical responses to protocols developed for either primary tumors or metastases [23].

Therefore, to find more effective and safe treatments, pharmacological studies of substances isolated from plants, as well as synthetic derivatives based on these natural compounds, have intensified [24,25]. Despite its relatively common usage and several pharmacological evaluations, studies describing the potential antiproliferative of E. uniflora are scarce. Therefore, the objective of this study was to perform detailed chemical analysis and in vitro evaluation of the antiproliferative effects of extracts and isolated compounds from the leaves of E. uniflora.

2. MATERIALS AND METHODS

2.1 Solvents and Reagents

All solvents and reagents were of analytical grade and the water was distilled and deionized. The solvents used were ethyl acetate, dichloromethane, ethanol and hexane (Vetec®, Rio de Janeiro, Brazil). An HPLC was used for the chromatographic analysis (Varian® Pro-Star) with automatic injector (20 μl), ternary gradient
pump, UV/Vis detector and a reversed-phase C-18 (250 x 4.5 mm) Kromasil® column (Sigma-Aldrich®), ODS (5 μm).

2.2 Plant Material

Leaves of *E. uniflora* were collected in Chapecó (SC), Brazil (27°05'33.4"S 52°39'54.3"O) in September of 2017. The plant samples were authenticated by Osmar dos Santos Ribas, curator of Herbarium of the Municipal Botanical Museum Curitiba (PR) where a voucher specimen (# 316818) was deposited.

2.3 Preparation of Extracts of Eugenia uniflora

The leaves of *E. uniflora* were dried for 10 days at room temperature (25 ± 5°C) and the plant material of the same particle size was collected by passage through a mesh (425 μm; 35 Tyler/Mesch). The extracts were produced utilized the dried plant material of *E. uniflora* (10 g) and dichloromethane (200 ml) by maceration for 5 days. The resulting vegetable residues of extraction (marc) were dried at ambient temperature and after extracted by maceration in ethanol (70%, 200 ml, v/v) for 5 days. After Büchner filtration, both extracts were filtered, concentrated by rotary evaporation under reduced pressure, lyophilized, weighed, identified, and stored in a freezer at -20°C. Subsequently, aliquots of dichloromethane (DEE) and hydroethanolic (HEE) extracts were used for biological and chemical analysis.

2.4 Chemical Analysis

2.4.1 Total phenolic content

The determination of the total phenol content of HEE was performed according to Sousa et al. [26]. Briefly, an aliquot (100 μl) of HEE (1 mg/ml in MeOH) was made up to 3 ml with distilled water, thoroughly mixed with 0.5 ml of Folin-Ciocalteu reagent for 3 min, followed by the addition of 2 ml of 20% (w/v) sodium carbonate. Finally, the volume (solution) was adjusted to 10 ml with distilled water. The mixture was allowed to stand for 60 min in the dark. Absorbance was measured at 750 nm using MeOH as control. The total phenolic content was calculated from the calibration curve produced with gallic acid standard (2.5 to 50 μg/ml), and the results were expressed as mg of gallic acid equivalents per g of extract (n = 3).

2.4.2 Total flavonoid content

The total flavonoid content of the crude extract was determined by the aluminum chloride colorimetric method [27]. A sample (1 g) of leaves of *E. uniflora* (particle size: 425 μm) was mixed with 75 ml of MeOH:H₂O:AcOH solution (140:50:10 v/v). The mixture was extracted under reflux for 30 min. After filtration, the filtrate was made up to 100 ml with the same solvent. An aliquot (5 ml) was homogenized with 2.5 ml of aluminum chloride solution (0.5 g of aluminum chloride and 0.1 g of sodium acetate diluted in 100 ml of MeOH). The solution stayed in the dark and after 30 min, the absorbance was measured at 425 nm. The results were expressed as mg quercetin/100 g dry plant material (n = 6).

2.4.3 HPLC analysis

Chromatographic analyses were performed using the method of Hoffmann-Ribani & Rodriguez-Amaya [28]. HEE was partitioned on a separating funnel with solvents of increasing polarity (hexane, chloroform, ethyl acetate, and n-butanol). The EtOAc fraction (10 mg/ml) was subjected to solid phase extraction on a Phenomenex® Stracta C18-E SPE cartridge (500 mg/3 ml) and used as an eluent for cleaning 5% MeOH (v/v) and MeOH 100% in extraction. Chromatograms were obtained on a Varian® ProStar with automatic injection (20 μl), ternary pump gradient, UV/Vis detector, and Kromasil® ODS (5 μm) reverse phase C-18 column (250 x 4.5 mm) at 24°C ± 2°C. A two-solvent system was used, comprising MeOH (solvent A) and H₂O, 0.3% v/v with HCO₂H (solvent B). The solvent gradient was 20% A for 6 min, 52% A for 15 min, 72% A for 27 min, and 10% A for 30 min. The flow rate was 0.8 ml/min. Detection was performed at 370 nm and an authentic external standard with known retention times, followed by UV spectrum, was used. For the production of calibration curves, methanolic solutions of quercetin standard (anhydrous Sigma-Aldrich®), St. Louis, Missouri, USA) at concentrations of 3.12, 6.25, 12.5, 25, and 50 μg/ml were analyzed in, triplicate. All extracts and solvents were filtered through Micropore® filters (0.45 μm) before the chromatographic analysis.

2.4.4 NMR analysis

1D and 2D NMR experiments were acquired in CDCl₃ at 303 K on a Bruker AVANCE III 600 NMR spectrometer, observing ¹H at 600.13 MHz and ¹³C 150.91 MHz. One-bond and long-range ¹H-¹³C correlation from HSQC and HMBC NMR
experiments were optimized for average coupling constants $^{1}J(H,C)$ and $^{3}J(H,C)$ of 140 and 8 Hz, respectively. All $^{1}H$ and $^{13}C$ NMR chemical shifts are given in ppm (5), using tetramethylsilane as internal reference, with coupling constants (J) in Hz.

2.4.5 Chemical isolation

DEE (1.71 g) was dissolved in n-hexane, mixed with silica gel, and subjected to liquid column chromatography using a stationary phase of silica gel (Merck®, Darmstadt, Germany) and eluted with a solution of n-hexane and EtOAc (90:10 v/v) increasing in polarity to 90% EtOAc (v/v). The subfractions (n = 10.5 ml each) were collected by similarity through thin layer chromatography (TLC) with a mobile phase of n-hexane: EtOAc (80:20 v/v) and the subsequent detection at 366 nm. Subfraction 6 (0.038 g) yielded an isolated compound (Compound 1).

2.4 In Vitro Antiproliferative Assay

The antiproliferative effect of the HEE, DEE, and compound 1 was investigated by using the protocol described by Monks et al. [29]. A panel of nine human cancer cell lines [U-251 (glioma, CNS), MCF-7 (breast), NCI-ADR/RES (ovarian cell expressing a multiple drugs resistance phenotype), 786-0 (kidney), NCI-H460 (lung, non-small cell), PC-3 (prostate), OVCAR-03 (ovarian), HT-29 (colon adenocarcinoma), and K-562 (chronic myeloid leukemia)], provided by Frederick Cancer Research & Development Center, National Cancer Institute, Frederick, MA, USA and one immortalized human cell line (HaCat, keratinocyte), provided by Dr. Ricardo Della Coletta (University of Campinas), were used.

Stock and experimental cultures were grown in 5 ml RPMI-1640 supplemented with 5% fetal bovine serum (RPMI/FBS 5%) and penicillin/streptomycin (1000 U/ml and 1000 μg/ml (1 ml/l; RPMI-1640). Stock solutions of the samples (5 mg) were prepared in DMSO (50 μl), followed by successive dilutions in RPMI/FBS 5% to give final concentrations of 0.25, 2.5, 25, and 250 μg/ml. Doxorubicin was used as a positive control at final concentrations of 0.25, 2.5, 25, and 250 μg/ml. Cells were seeded in 96-well plates (100 μl cells/well, density: 3–7 × 10⁴ cells/ml) and incubated with each concentration of sample solution or doxorubicin (100 μl/well) for 48 h at 37°C in an atmosphere of 5% CO₂. Each test was performed in triplicate (n = 3). Before (T0 plate) and after (T1 plates) sample addition, cells were fixed with 50% trichloroacetic acid (50 μl well) and stained with sulforhodamine B. The absorbance of the cells was measured at 540 nm to quantitate cell proliferation. The GI₅₀ (concentration that produces 50% cell growth inhibition or cytostatic effect) and the TGI (concentration that resulted in total cellular growth inhibition) values were determined from non-linear regression applied to a sigmoidal curve computed by using Origin 8.0 software (OriginLab Corporation).

3. RESULTS

3.1 Total Phenolic and Flavonoids Content

The Table 1. presents the total phenolic content of HEE, calculated through of calibration curve (y = 0.0351x + 0.0063; $R^2$ = 0.9997) and represented per gallic acid equivalents/g. The total flavonoid content in leaves of E. uniflora, was considered elevated and represented by mg of quercetin/100 g of plant material.

3.2 HPLC Analysis and Chemical Isolation of HEE

The chromatogram of the EtOAc fraction and HEE (370 nm) is shown in Fig. 1. The presence of quercetin was detected (RT = 20.6 min). From the equation of the line (y = 12.085x + 0.0063; $R^2$ = 0.9997), the concentration of quercetin in the EtOAc fraction and HEE was 8.28 and 34.08 mg/g, respectively.

Compound 1 was isolated from DEE by chromatographic fractionation. The compound was identified as β-sitosterol by comparison of the experimental spectra ($^{1}H$ NMR, $^{13}C$ NMR) with those previously described [30].

<table>
<thead>
<tr>
<th>Table 1. Total phenolics and flavonoids content from Eugenia uniflora</th>
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<tbody>
<tr>
<td>Total phenolic or flavonoid content</td>
</tr>
<tr>
<td>-------------------------------------</td>
</tr>
<tr>
<td>Total phenolic content$^a$</td>
</tr>
<tr>
<td>Total flavonoid content$^b$</td>
</tr>
</tbody>
</table>

$^a$ mg gallic acid equivalents (GAE)/g of hydroethanolic extract (HEE)  
$^b$ quercetin: mg/100 g plant material (leaves)
Fig. 1. HPLC profile for the EtOAc fraction obtained from the hydroethanolic extract of leaves from Eugenia uniflora (HEE; 10 mg/ml in MeOH): (a). Presence of quercitin on EtOAc fraction subjected to solid-phase extraction; (b). Quercitin standard (12.5 µg/ml in MeOH) (Rf: 20.6 min)

β-Sitosterol (5-Stigmasten-3β-ol) (1): White crystal; 1H-NMR (400.13 MHz, CDCl3): δ 5.35 (m, 1H, H-6), 3.51 (tdd, 1H, H3), 0.98 (s, 3H, H-19), 0.92 (d, 3H, H-21, J = 6.5 Hz), 0.85 (t, 3H, H-29, J = 5.6 Hz), 0.86 (d, 3H, H-26, J = 6.9 Hz), 0.82 (d, 3H, H-27, J = 6.9 Hz), 0.68 (s, 3H, H-18); 13C-NMR (100.61 MHz, CDCl3): δ 140.8 (C-5), 121.7 (C-6), 71.8 (C-3), 56.8 (C-14), 56.1 (C-17), 50.2 (C-8), 45.2 (C-24), 42.4 (C-13), 42.3 (C-4), 39.8 (C-12), 37.3 (C-1), 36.5 (C-10), 36.2 (C-20), 34.0 (C-22), 31.9 (C-8), 31.9 (C-7), 31.7 (C-2), 29.2 (C-25), 28.3 (C-16), 26.2 (C-23), 24.3 (C-15), 23.1 (C-28), 21.1 (C-11), 19.4 (C-19), 19.1 (C-27), 18.9 (C-26), 18.8 (C-21), 12.0 (C-18), 11.9 (C-29).

3.3 Antiproliferative Effects

The antiproliferative effects of the extracts and the isolated compound of E. uniflora are shown
in Fig. 2. HEE and DEE extracts resulted in different growth inhibition profiles. DEE was more effective than HEE, inhibiting growth of all tumor cell lines tested, with the strongest effect on OVCAR-3 cells (GI$_{50}$: 8.45 and TGI = 51.29 μg/ml) (Fig. 2 and Table 2). In addition, β-sitosterol, isolated from DEE, showed potential antiproliferative activity, completely inhibiting the proliferation of U-251 cells when applied at 7.37 μg/ml (Fig. 3 and Table 3).

4. DISCUSSION

Natural products have become important sources of anticancer agents. Moreover, novel natural compounds with several structures, isolated from plant sources, have been developed as prototypes and their subsequent structural modification has afforded compounds with pharmacological potential [31]. In this study, extracts of high and low polarity were prepared from the leaves of *E. uniflora* (HEE and DEE), and phenolic, flavonoids and quercetin compounds were detected in HEE. These results corroborate previous studies where the presence of quercetin has been reported [17].

However, by using a solid-phase extraction technique, this is the first study to identify and quantify quercetin by HPLC, which contributes to quality control studies of plant extracts. The β-sitosterol isolated from DEE was previously described by Samy et al. [32], in the EtOAc fraction obtained from the methanolic extract of *E. uniflora* leaves. The flavonoids and steroids found in *E. uniflora* have been documented as natural bioactive products with potent anticancer activity [33,34].

![Fig. 2. Antiproliferative effect *in vitro* from *Eugenia uniflora*. (a): hydroethanolic extract (HEE). (b): dichloromethane extract (DEE)](image)

*Note: Concentration range: 0.25 - 250 μg/ml; exposition time: 48 h; human tumor cell lines: glioblastoma (U-251), breast (MCF-7), ovarian expressing the resistance phenotype (NCI/ADR-RES), 786-O (kidney), non-small cells lung (NCI-H460), prostate (PC-3), ovarian (OVCAR-3), colon (HT-29), leukemia (K-562); human immortalized cell line: keratinocytes (HaCat)*
Table 2. GI<sub>50</sub> and TGI values for Hydroethanolic (HEE) and Dichloromethane (DEE) extracts of Eugenia uniflora against different cell lines

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>HEE GI&lt;sub&gt;50&lt;/sub&gt; (µg/ml)</th>
<th>DEE GI&lt;sub&gt;50&lt;/sub&gt; (µg/ml)</th>
<th>HEE TGI (µg/ml)</th>
<th>DEE TGI (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-251</td>
<td>27.06</td>
<td>27.08</td>
<td>170.25</td>
<td>81.37</td>
</tr>
<tr>
<td>MCF-7</td>
<td>30.50</td>
<td>25.25</td>
<td>161.15</td>
<td>99.44</td>
</tr>
<tr>
<td>NCI/ADR-RES</td>
<td>65.16</td>
<td>27.31</td>
<td>a</td>
<td>191.48</td>
</tr>
<tr>
<td>786-0</td>
<td>65.78</td>
<td>25.30</td>
<td>a</td>
<td>66.46</td>
</tr>
<tr>
<td>NCI-H460</td>
<td>37.50</td>
<td>25.92</td>
<td>a</td>
<td>79.35</td>
</tr>
<tr>
<td>PC-3</td>
<td>88.84</td>
<td>27.24</td>
<td>a</td>
<td>82.65</td>
</tr>
<tr>
<td>OVCAR-3</td>
<td>56.46</td>
<td>8.45</td>
<td>a</td>
<td>51.29</td>
</tr>
<tr>
<td>HT-29</td>
<td>43.96</td>
<td>28.18</td>
<td>a</td>
<td>77.68</td>
</tr>
<tr>
<td>K-562</td>
<td>130.21</td>
<td>26.24</td>
<td>a</td>
<td>99.54</td>
</tr>
<tr>
<td>HaCaT</td>
<td>30.23</td>
<td>26.24</td>
<td>223.77</td>
<td>91.44</td>
</tr>
</tbody>
</table>

Note: GI<sub>50</sub> = 50% growth inhibition; TGI = total inhibition of growth; a Effective concentration higher than the highest tested concentration (250 µg/ml)

Fig. 3. Antiproliferative effect in vitro of β-sitosterol isolated of dichloromethane extract from Eugenia uniflora

Note: Concentration range: 0.25 - 250 µg/ml; exposition time: 48 h; human tumor cell lines: glioblastoma (U-251), breast (MCF-7), ovarian expressing the resistance phenotype (NCI/ADR-RES), 786-O (kidney), non-small cells lung (NCI-H460), prostate (PC-3), colon (HT-29), leukemia (K-562); human immortalized cell line: keratinocytes (HaCaT)

In accordance with guidelines of the National Cancer Institute (NCI), extracts or molecules are considered active if they are able to inhibit cell proliferation by 50% at concentrations less than 30 µg/ml (GI<sub>50</sub> < 30 µg/ml) [35, 36]. According to this criterion, HEE was inactive in this study, whereas DEE showed potential antiproliferative against ovarian and breast tumor cells line. There are a few previous reports of the antiproliferative effects of E. uniflora. However, when using increased concentrations (50 and 100 µg/ml), Dernardin et al. [37], observed the antiproliferative activity of hydroethanolic extract of the fruits of E. uniflora, and described early and late apoptotic effects of the extract on hepatic stellate cells (GRX) by using flow cytometry. Dernardin et al. also reported a decrease in mitochondrial membrane potential and mitochondrial membrane protein content, and demonstrated that the reduction in cell proliferation was dose-dependent.

In this study, HEE showed antiproliferative activity against breast tumor cells (MCF-7; GI<sub>50</sub>: 30.50 µg/ml). Similar results were obtained by Li et al. [38] in a luminesce-based cell viability assay, which showed antiproliferative activity of the methanolic extract of the fruits of Eugenia jambolana Lam., due to the induction of apoptosis in MCF-7 and MDA-MB-231 strain breast tumor cells (GI<sub>50</sub> of 27 and 40 µg/ml, respectively).
In contrast, the dichloromethane extract of *E. uniflora* (DEE) showed potential antiproliferative effects against all tested strains (GI$_{50}$ < 30 µg/ml). The higher antiproliferative activity of the low-polarity dichloromethane extracts, compared with the constituents present in less polar extracts, as well as the higher affinity of these molecules, the higher-polarity hydroethanolic extracts is likely due to the more lipophilic chemical across cell membranes [39].

To investigate the antiproliferative activity by a bioassay-guided process, DEE was fractionated on chromatographic column, resulting in the isolation of β-sitosterol, which was subsequently evaluated. β-Sitosterol showed reduced values of GI$_{50}$ against all strains tested, especially for glioblastoma (U-251). According to Fouche et al. [40], molecules that demonstrate total inhibition values of cell growth (TGI) at between 6.25 and 15 µg/ml are considered to have moderate antiproliferative activity, and TGI values of < 6.25 µg/ml are considered to exert potent antiproliferative activity. Thus, β-sitosterol was considered to have moderate antiproliferative activity against glioblastoma and leukemia (K-562 cells) (TGI: 7.37 and 10.94 µg/ml, respectively), and no effect on normal cells (HaCat), which indicated the high selectivity of the compound. The reduced selectivity of many chemotherapeutic agents causes damages to normal cells, resulting in several side effects. Therefore, it is desirable to find new chemotherapeutic drugs that are selective to tumor cells [35]. β-Sitosterol showed a high selectivity index (SI), as calculated by the ratio of cell death between HaCat cells (nontumor cells) and tumor cells (CC$_{50}$/GI$_{50}$). This showed that the molecule was more active against glioma (CNS) and leukemia tumor cells (SI: 16.5 and 2.62, respectively), and less active against healthy cells.

This is the first report to describe the antiproliferative activity of β-sitosterol (BS) against glioblastoma and leukemia tumor lines. However, through *in vitro* and *in vivo* studies it has been suggested that sterols (β-sitosterol, campesterol, and stigmasterol) have protective abilities against colon, prostate, and breast tumors [41]. Chai, Kuppusamy & Kanthimathi [42] reported that β-sitosterol could inhibit the proliferation of MCF-7 cells in a dose-dependent manner owing to the presence of estrogenic receptors involved in breast cancer. Against a prostate cancer cell line (decrease in cell growth of 24%) and four-fold induction of apoptosis, which was followed by “rounding up” of the cells, an enhancement in ceramide production was found to occur by BS (16 µM), and these effects have been promoted by activation of the sphingomyelin cycle [43].

Regarding the pharmacological mechanism, several studies have indicated that BS inhibits the growth of various cancer cell lines in culture that are associated with the activation of the sphingomyelin cycle, cell cycle arrest [41], and the stimulation of apoptotic cell death [44]. In a review, Ovesna, Vachalkova & Horvathova [45] reported the experimental inhibition of colon and breast cancer development by β-sitosterol. It was reported that this compound could have different effects on tumors, such as inhibitory effects on the development, promotion, and induction of cancerous cells, as well as the inhibition of tumor cell invasion and metastasis. It is likely that in this study, the highly selective action of β-sitosterol isolated from DEE caused similar cellular events as part of its action against glioblastoma and leukemia cell lines.

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>β-sitosterol GI$_{50}$ (µg/ml)</th>
<th>β-sitosterol TGI (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-251</td>
<td>0.40</td>
<td>7.37</td>
</tr>
<tr>
<td>MCF-7</td>
<td>3.46</td>
<td>19.64</td>
</tr>
<tr>
<td>NCI/ADR-RES</td>
<td>2.07</td>
<td>193.80</td>
</tr>
<tr>
<td>786-0</td>
<td>21.88</td>
<td>64.15</td>
</tr>
<tr>
<td>NCI-H460</td>
<td>2.83</td>
<td>45.46</td>
</tr>
<tr>
<td>PC-3</td>
<td>20.89</td>
<td>22.94</td>
</tr>
<tr>
<td>HT-29</td>
<td>29.82</td>
<td>114.90</td>
</tr>
<tr>
<td>K-562</td>
<td>2.71</td>
<td>10.94</td>
</tr>
<tr>
<td>HaCat</td>
<td>6.59</td>
<td>a</td>
</tr>
</tbody>
</table>

*Note: GI$_{50}$ = 50% growth inhibition; TGI = total inhibition of growth; *$^a$Effective concentration higher than the highest tested concentration (250 µg/ml)*
5. CONCLUSION
Phenolic compounds are present in the leaves of *E. uniflora*. In the ethyl acetate fraction, quercetin was detected by HPLC analysis. β-Sitosterol isolated from the dichloromethane extract of *E. uniflora* exhibited promising antiproliferative effects *in vitro*, especially against glioblastoma and leukemia cell lines.

CONSENT
It is not applicable.

ETHICAL APPROVAL
It is not applicable.

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COMPETING INTERESTS
Authors have declared that no competing interests exist.

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