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Styrax camporum and *S. ferrugineus* fruits: norneolignans, antioxidant and cytotoxic activities

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ABSTRACT

The present study evaluated the antioxidant and cytotoxic activities of the methanol extract of *Styrax camporum* and *S. ferrugineus* fruits. Purification of both extracts, which displayed weak antioxidant and cytotoxic activities, resulted in isolation of seven compounds: homoegonol (**1**), egonol (**2**), demethoxy egonol-2-methylbutanoate (**3**), egonol gentiobioside (**4**), demethoxy egonol (**5**), demethoxy homoegonol (**6**), and egonol-2-methylbutanoate (**7**). XTT cell culture and DDPH assays helped to assess the activity of the pure compounds. Compared to gallic acid at 66.7 µg/mL, the evaluated *nor*neolignans were inactive in the DPPH assay. The cytotoxicity assay revealed that egonol acetate and compound **3** had CC_{50} of 267.90 and 19.10 µg/mL at 24 h. Compound **3** was also assayed on cancer cells lines (HeLa, MO59J and MCF-7), but it did not reduce the viability of these cells with the same efficiency. Again, the results presented here confirmed that the egonol core is a promising structural feature for anticancer drug research.

INTRODUCTION

A diet that advocates fruits intake has long been recognized as an ally in promoting health and preventing disease. Such diet reduces the risk of chronic diseases such as cancer, diabetes, cardiovascular disease, and arthritis. Indeed, fruits are normally rich in antioxidants, like tannins, stilbenes, flavonoids, and phenolic acids (Kozłowska and Szostak-Wegierek, 2014; Zhang *et al.*, 2015). Several fruits with high potential for agriculture are native to the Brazilian cerrado. Local people have traditionally consumed these fruits, which display high nutritional and economic potential (Siqueira *et al.*, 2013; Agostini-Costa *et al.*, 2006). In fact, the nutritional value of these fruits has led to their increasing consumption nationalwide and worldwide (Rufino *et al.*, 2010). In addition, these fruits present high antioxidant capacity and prominent phenolic content (Cândido *et al.*, 2015; Siqueira *et al.*, 2013; Rocha *et al.*, 2011; Silva *et al.*, 2008). *Styrax camporum* Pohl and *Styrax ferrugineus* Ness et. Mart. are two species that occur in the Brazilian cerrado. However, no research on the chemical and biological potential of their fruits has been conducted. Existing studies on *S. camporum* and *S. ferrugineus* have been limited to their leaves and barks (Francielli de Oliveira *et al.*, 2012; Braguine *et al.*, 2012; Teles *et al.*, 2005; Bacchi and Sertié, 1995; Pauletti *et al.*, 2000). In indigenous medicine, *S. camporum* and *S. ferrugineus* have been employed in the treatment of gastrointestinal diseases and fever, respectively (Lorenzi, 1982; Rodrigues and Carvalho, 2008). The two species produce a resinous material that is secreted when the barks and trunks are injured by sharp objects, and this material can be used in place of benzoin resin, which presents anti-inflammatory actions (Silva-Júnior and Pereira, 2009; de Almeida *et al.*, 1998). Additionally, the fruits of *S. ferrugineus* are used in homemade jams (Silva-Júnior, 2012). Furthermore, the fruits of *S. camporum* feed also the wild animals, and have slightly sweet taste (Silva-Junior and Pereira, 2009; Kuhlmann 2012).

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Recently, LC-MS analysis of the fruits of *S. ramirezii*, from Mexico, accomplished by Timmers *et al.* (2015) has enabled their chemical characterization and determination of their antioxidant and anti-inflammatory activities. These authors also evaluated the compounds isolated from these fruits, egonol and homoegonol, in the bioassays. The results suggested that the inclusion of *S. ramirezii* fruits in the diet contributes with antioxidant and anti-inflammatory compounds intake.

Considering the need to understand native fruits better, we have assayed the methanol crude extract of *Styrax camporum* and *S. ferrugineus* fruits in the presence of DPPH (2,2-diphenyl-1-picrylhydrazyl) and evaluated their cytotoxicity. We have also investigated the chemical composition and antioxidant and cytotoxicity properties of the isolated compounds.

MATERIAL AND METHODS

General

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker AVANCE DRX 500 or on a Bruker DPX-400 spectrometer (Bruker Corporation, USA); TMS was used as internal standard. HPLC was accomplished on a Shimadzu LC-6AD system (Kyoto, Japan) equipped with a DGU-20A5 degasser, an SPD-20A series UV-VIS detector or an SPDM-20A series PDA detector, a CBM-20A communication bus module, a Reodyne manual injector, and LCsolution software. The columns and pre-columns were SHIMADZU Shim-pack ODS (particle diameter 5 µm, 250 x 4.60 mm, and 250 x 20 mm). The MeOH employed in the analyses was HPLC grade, J. T. Baker. Direct-Q UV3 system, Millipore was used to obtain ultrapure water. Silica gel 60 (230-400 mesh, Sigma-Aldrich) was employed for column chromatography, and silica on TLC Al foils with fluorescent indicator 254 nm (Sigma-Aldrich) was also used. Prep-TLC was conducted on silica gel type G (Sigma-Aldrich).

Fruit Material

The fruits of *Styrax camporum* Pohl were collected at the campus of the University of São Paulo, Pirassununga, SP, Brazil, in February 2013, and the fruits of *S. ferrugineus* Ness et. Mart. were collected at Santa Cecilia, Patrocínio Paulista, SP, Brazil, in October 2012. The materials were identified by Prof. V.M.M. Gimenez and Prof. A.R.B. Araújo. Voucher specimens (SPFR 12170 and SPFR 12169, respectively) were deposited in the Herbarium of the Department of Biology, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, University of São Paulo, Brazil (Herbarium SPFR).

Extraction and isolation

The air-dried, powdered fruits (94.7 g) of *S. ferrugineus* were extracted with AcOEt, and then with MeOH, yielding 8.8 and 8.6 g of crude extract, respectively. The solvents were selected based on previous work (Liu *et al.*, 2011). The crude MeOH extract (MESF, 3.5 g) was fractionated on a column chromatography on silica gel using a gradient of *n*-hexane/EtOAc as eluent, which afforded 66 fractions. In a previous study, our research group had purified the fractions 19-30 and 32-36 and obtained compounds **1** and **2** (de Oliveira *et al.*, 2016). Fractions 12-13 yielded compound **3** (12.9 mg) after Prep-TLC using *n*-hexane-EtOAc (9:1, v/v) as the mobile phase. Fraction 65 was purified by Prep-HPLC using MeOH/H_2O (60:40, v/v) as mobile phase, UV detection at 254 nm, and 8 mL/min flow rate were used, to yield five fractions. Fraction 3 gave compound **4** (54.8 mg, t_R 43.9 min).

The air-dried, powdered fruits (63 g) of *S. camporum* were extracted with MeOH, yielding 13.3 g of crude extract. The crude MeOH extract (MESC, 5 g) was fractionated on a column chromatography on silica gel (70–230 mesh) by using a gradient of *n*-hexane/EtOAc as eluent, which afforded 59 fractions. Fractions 26-29, 30-32 and 43-46 furnished compounds **5**, **1** and **2**, respectively.

Fractions 33-39 were purified by using Prep-HPLC with MeOH/H_2O (80:20, v/v) as eluent, UV detection at 254 nm, and 8 mL/min flow rate, to yield three fractions. Fraction 2 gave compound **6** (10 mg, t_R 13.1 min). Fractions 13-19 were purified by using Prep-HPLC with MeOH/H_2O (85:15, v/v) as eluent, UV detection at 254 nm, and 8 mL/min flow rate, to yield nine fractions. Fraction 6 gave compound **7** (10.3 mg, t_R 36.7 min).

Homoegonol (1).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.46 (1H, dd, *J*= 8.2 and 1.8, H-6′), 7.37 (1H, d , *J*= 1.8, H-2′), 6.98 (1H, br s, H-4), 6.93 (1H, d, *J*= 8.2, H-5′) 6.84 (1H, s, H-3), 6.64 (1H, br s, H-6), 4.04 (3H, s, 7-OCH_3), 3.99 (3H, s, 4′-OCH_3) 3.93 (3H, s, 3′-OCH_3), 3.71 (2H, t, *J*= 6.0, H-3″), 2.79 t (2H, *J*= 7.9, H-1″), 1.96 (2H, m, H-2″).

Egonol (2).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.40 (1H, dd, *J*= 8.0 and 1.7, H-6′), 7.32 (1H, d, *J*= 1.7, H-2′), 6.97 (1H, d, *J*= 1.3, H-4), 6.87 (1H, d, *J*= 8.0, H-5′), 6.79 (1H, s, H-3), 6.63 (1H, d, *J*= 1.3, H-6), 6.01 (2H, s, OCH_2O), 4.03 (3H, s, 7-OCH_3), 3.71 (2H, t, *J*= 6.3, H-3″), 2.78 (2H, t, *J*= 7.5, H-1″), 1.94 (2H, m, H-2″).

7-Demethoxy egonol-2-methylbutanoate (3).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.32 (1H, d, *J*= 8.2, H-7), 7.30 (1H, dd, *J*= 8.0 and 1.7, H-6′), 7.26 (1H, d, *J*= 1.3, H-4), 7.23 (1H, d, *J*= 1.7, H-2′), 7.00 (1H, dd, *J*= 8.2 and 1.3, H-6), 6.81 (1H, d, *J*= 8.0, H-5′), 6.73 (1H, s, H-3), 5.94 (2H, s, OCH_2O), 4.04 (2H, t, *J*= 6.0, H-3″), 2.70 (2H, t, *J*= 7.9, H-1″), 2.30 (1H, m, H-2a), 1.92 (2H, m, H-2″), 1.61 (1H, m, H-3a), 1.41 (1H, m, H-3a), 1.07 (3H, d, *J*= 7.0, H-5a), 0.83 (3H, t, *J*= 7.4, H-4a).^{13}C NMR (100 MHz, $CDCl_3$): δ 176.8 (C=O, C-1a), 156.0 (C-2), 153.4 (C-8), 148.1 (C-4′), 148.0 (C-3′), 135.9 (C-5), 129.5 (C-9), 124.8 (C-1′), 124.5 (C-6), 120.0 (C-4), 119.1 (C-6′), 110.7 (C-7), 108.7 (C-5′), 105.4 d (C-2′), 101.3 (OCH_2O), 100.0 (C-3), 63.5 (C-3″), 41.1

(C-2a), 32.1 (C-1″), 30.9 (C-2″), 26.8 (C-3a), 16.7 (C-5a), 11.7 (C-4a).

Egonol gentiobioside (4).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 7.40 (1H, dd, *J*= 8.0 and 1.5, H-6′), 7.43 d (1H, d, *J*= 1.5, H-2′), 7.00 (1H, br s, H-4), 7.21 (1H, s, H-3), 7.03 (1H, d, *J*= 8.0, H-5′), 6.78 (1H, br s, H-6), 6.09 (2H, s, OCH_2O), 4.21 (1H, d, *J*= 7.5, H-1‴), 4.12 (1H, d, *J*= 7.9, H-1‴), 3.94 (3H, s, 7-OCH_3), 3.92 (1H, d, *J*= 10.4, H-6‴), 3.79 (1H, m, H-3″), 3.68 (1H, dd, *J*= 11.2 and 5.3, H-6⁗), 3.54 (1H, dd, *J*= 10.4 and 6.4, H-6⁗), 3.43 (1H, m, H-3″), 3.27 (2H, m, H-4⁗ and H-5⁗), 3.14 (2H, t, *J*= 8.5, H-5‴ and H-3⁗), 3.08 (2H, t, *J*= 8.5, H-3‴ and H-4⁗), 2.98 (3H, m, H-6⁗, H-2‴ and H-2⁗), 2.72 (2H, m, H-1″), 1.86 (2H, m, H-2″).^{13}C NMR (125 MHz, DMSO-d_6): δ 156.0 (C-2), 149.0 (C-4′), 148.0 (C-3′), 145.2 (C-7), 142.0 (C-8), 138.0 (C-5), 131.0 (C-9), 125.0 (C-1′), 119.6 (C-6′), 113.0 (C-4), 109.7 (C-5′), 108.7 (C-6), 105.8 (C-2′), 104.9 (C-1⁗), 103.7 (C-1‴), 102.3 (OCH_2O), 102.0 (C-3), 77.5 (C-3‴, C-5‴ and C-3⁗), 76.6 (C-5⁗), 74.2 (C-2‴ and C-2⁗), 70.8 (C-4‴), 70.5 (C-4⁗), 69.2 (C-6‴), 68.7 (C-3″), 66.5 (C-6⁗), 56.6 (7-OCH_3) 32.7 (C-1″), 32.3 (C-2″).

*Demethoxy egonol (**5**).*

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.37 (1H, d, *J*= 8.0, H-7), 7.35 (1H, d, *J*= 8.0, H-5′), 7.34 (1H, d, *J*= 1.6, H-4), 7.28 (1H, d, *J*= 1.6, H-2′), 7.07 (1H, br d, *J*= 8.0, H-6), 6.86 (1H, dd, *J*=8.0 and 1.6, H-6′), 6.77 (1H, s, H-3), 5.98 (2H, s, OCH_2O), 3.66 (2H, t, *J*= 6.2, H-3″), 2.77 (2H, t, *J*= 7.2, H-1″), 1.91(2H, m, H-2″). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.9 (C-2), 153.3 (C-8), 148.1 (C-3′), 148.0 (C-4′), 136.4 (C-5), 129.5 (C-9), 124.8 (C-1′), 124.6 (C-6), 120.0 (C-4), 119.0 (C-5′), 110.7 (C-7), 108.6 (C-6′), 105.4 (C-2′), 101.3 (O-CH_2-O), 100.0 (C-3), 62.1(C-3″), 34.7 (C-2″), 31.9 (C-1″).

Demethoxy homoegonol (6).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.41 (1H, dl, *J*= 8.0, H-6), 7.41 (1H, br d, *J*= 8.0, H-6′), 7.36 (1H, br s, H-4), 7.36 (1H, br s, H-2′), 7.10 (1H, d, *J*= 8.0, H-7), 6.93 (1H, d, *J*= 8,0, H-5′), 6.85 (1H, s, H-3), 3.99 (3H, s, 4′-OCH_3), 3.93 (3H, s, 3′-OCH_3), 3.70 (2H, t, *J*= 6.2, H-3″), 2.80 (2H, t, *J*= 7.5, H-1″), 1.94 (2H, m, H-2″). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.0 (C-2), 153.4 (C-8), 149.5 (C-4′), 149.1 (C-3′), 136.4 (C-5), 129.6 (C-9), 124.6 (C-1′), 124.5 (C-6), 119.9 (C-4), 117.9 (C-6′), 111.3 (C-5′), 110.7 (C-7), 107.9 (C-2′), 99.8 (C-3), 62.3 (C-3″), 56.0 (O-CH_3), 34.7 (C-1″), 32.0 (C-2″).

Egonol-2-methylbutanoate (7).

^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.40 (1H, dd, *J*= 8.0 and 2.0, H-6′), 7.32 (1H, d, *J*= 2.0, H-2′), 6.95 (1H, d, *J*= 1.7, H-4), 6.87 (1H, d, *J*= 8,0, H-5′), 6.79 (1H, s, H-3), 6.60 (1H, d, *J*= 1.7, H-6), 6.00 (2H, s, OCH_2O), 4.12 (2H, t, *J*= 6.7, H-3″), 4.03 (3H, s, 7-OCH_3), 2.75 (2H, t, *J*= 7.9, H-1″), 2.39 (1H, m, H-2a), 1.98 (2H, m, H-2″), 1.68 m (1H, m, H-3a), 1.49 (1H, m, H-3a), 1.16 (3H, d, *J*= 7.0, H-5a), 0.92 (3H, t, *J*= 7.3, H-4a).

Acetylation of (1) and (2)

Homogonol (**1**) and egonol (**2**) (5 mg) were dissolved in acetic anhydride (3 mL) and pyridine (3 mL). The mixture was allowed to react overnight. Then, cold H_2O (30 mL) was added, and the mixture was extracted with CH_2Cl_2 (2 x 15 mL), HCl 10% (2 x 15 mL), and H_2O (3 x 15 mL). Anhydrous sodium carbonate was added on to the organic phase, which was then filtered and concentrated under reduced pressure, to yield acetate derivatives **1a** and **2a**.

Homoegonol acetate (1a).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.46 (1H, dd, *J*= 2.0 and 8.4, H-6′), 7.37 (1H, d, *J*= 2.0, H-2′), 6.97 (1H, d, *J*= 1.2, H-4), 6.93 (1H, d, *J*= 8.4, H-5′), 6.84 s (1H, s, H-3), 6.61 (1H, d, *J*= 1.2, H-6), 4.13 (2H, t, *J*= 7.8, H-3″), 4.03 (3H, s, 7-OCH_3), 3.93 (3H, s, 4′-OCH_3), 3.83 (3H, s, 3′-OCH_3), 2.76 (2H, t, *J*= 7.5, H-1″), 2.07 (3H, s, 3″-$COOCH_3$), 2.01 (2H, m, H-2″).

Egonol acetate (2a).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.40 (1H, dd, *J*= 1.6 and 8.2, H-6′), 7.32 (1H, d, *J*=1.6, H-2′), 6.96 (1H, d, *J*= 1.1, H-4), 6.87 (1H, d, *J*= 8.2, H-5′), 6.79 (1H, s, H-3), 6.61 (1H, d, *J*= 1.1, H-6), 6.01 (2H, s, OCH_2O), 4.12 (2H, t, *J*= 6.5, H-3″), 4.01 (3H, s, 7-OCH_3), 2.75 (2H, t, *J*= 7.7, H-1″), 2.00 (2H, m, H-2″), 2.07 (3H, s, 3″-$COOCH_3$).

Cytotoxicity Assay

The cytotoxicity of the extracts (MESF and MESC) and compounds (**1a**, **2a**, **3**, **4**, **5**, **6** and **7**) was first assessed in the case of cell line GM07492A (normal human lung fibroblasts). Then, compound **3** was tested in the case of the cancer cell lines: human breast adenocarcinoma (MCF-7), human cervical adenocarcinoma (HeLa), and human glioblastoma (MO59J). The cell lines were maintained as monolayers in plastic culture flasks (25 cm^2) containing HAM-F10 plus DMEM (1:1; Sigma-Aldrich), with 10% fetal bovine serum (Nutricell), 2.38 mg/mL Hepes (Sigma-Aldrich), 0.01 mg/mL streptomycin (Sigma-Aldrich), and 0.005 mg/mL penicillin (Sigma-Aldrich), at 37°C and under humidified atmosphere with 5% CO_2. Cells from the fourth to the twelfth passage were used.

Cytotoxicity was measured by using the Colorimetric Assay *In Vitro* Toxicology, XTT Kit (Roche Diagnostics). Briefly, 1 x 10^4 cells were seeded in microplates containing100 µL of culture medium (1:1 HAM F10 + DMEM or DMEM alone) supplemented with 10% fetal bovine serum and concentrations of the tested extracts and compounds ranging from 1.25 to 2500 µg/mL. Negative (no treatment), solvent (0.02% DMSO) and positive (25% DMSO) controls were added to the microplate. After incubation at 36.5 °C for 24 h, the culture medium was removed and the cells were washed with 100 µL of PBS. Next, 100 µL of culture medium HAM-F10 without phenol red was added. Then, 25 µl of XTT were plated and incubated at 36.5 °C for 17 h. The absorbance of the wells was determined at a wavelength of 450 nm and a reference length of 620 nm by using a

multi-plate reader (ELISA - Tecan - SW Magellan vs 5.03 STD 2P).

Antioxidant activity

The antioxidant activity of crude extracts (MESF and MESC) and compounds was determined by using DPPH (2,2-diphenyl-1-picrylhydrazyl). DPPH (26.7 µg/mL) dissolved in MeOH was added to the samples containing concentrations of the tested extracts/compounds ranging from 66.7 -1.67 µg/mL in MeOH. The mixture were incubated at room temperature, in the dark, for 30 min. Remaining DPPH was determined at 517 nm in a microplate reader. The antioxidant activity was expressed by using values obtained as percent of scavenging calculated from the equation: % of scavenging= (A_{DPPH} - A_{sample} /A_{DPPH})*100], where A_{DPPH} is the absorbance of the solution with DPPH only, and A_{sample} is the absorbance of the solution with extracts and compounds.

Statistical analyses

All the experiments were performed in triplicate, and the results are presented as the mean ± SD. The cytotoxicity effect was established based on the CC_{50} response parameter (cytotoxic concentration values that cause 50% cell growth inhibition for the different cell lines after 24h of observation) calculated with the GraphPad Prism program. One-way analysis of variance (ANOVA) was used to compare the differences between the means; significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Free radicals are recognized as key intermediaries in several diseases like diabetes mellitus, cancer, liver diseases, renal failure, and degenerative diseases, caused by a deficient natural antioxidant protection mechanism. Thus, researches for beneficial bioactivities from plants are considered to be a rational approach in drug development (Roy *et al.,* 2016). Additionally, fruits are known as super antioxidants and cancer protective agents, fruits are gaining attention for the exploration of their health improvement properties (Lim *et al.,* 2007; Saini *et al.,* 2014).

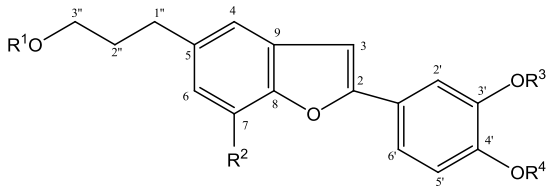
In this way, to establish the potential of the methanol crude extracts of *S. ferrugineus* (MESF) and of *S. camporum* (MESC) fruits, we screened the extracts for their antioxidant and cytotoxic activity *in vitro* (Table 1). The DPPH assay was selected due to its low-cost and easiness, and therefore has been used for the initial evaluation of the fruits extracts and isolated compounds antioxidant properties (Apak *et al.,* 2016). The percentage of DPPH scavenging was 18.47 and 2.40 at 66.7 µg/mL for MESC and MESF, respectively. MESC afforded better antioxidant results as compared to MESF. Regarding the DPPH assay, the values obtained for the extracts were considered weak as compared to the positive control, gallic acid. This result could be explained in terms of the extraction procedure: we did not use acidified methanol to extract the polyphenol compounds (Timmers *et al.,* 2015). In order to study the toxicological properties of the extracts we investigated the cytotoxicity on normal human lung fibroblast cells (GM07492A). The CC_{50} of MESC and MESF obtained in the assay was 270.8 and 164.7 µg/mL, respectively. According to Suffness and Pezzuto (1990), only CC_{50} lower than 30 µg/mL indicates cytotoxic action. Hence, neither of the tested extracts displayed cytotoxicity, but MESF was more active than the MESF.

Purification of MESF and MESC led to the isolation of seven compounds (Figure 1): homoegonol (**1**), egonol (**2**), demethoxy egonol-2-methylbutanoate (**3**), egonol gentiobioside (**4**), demethoxy egonol (**5**), demethoxy homoegonol (**6**), and egonol-2-methylbutanoate (**7**). NMR spectroscopy helped to establish their chemical structures, which agreed with published data (Segal *et al.,* 1967; Pauletti *et al.,* 2000; Akgul and Anil, 2003; Takanashi *et al.,* 1974; Lee *et al.,* 2008; Takanashi and Takizawa, 2002; Takanashi and Takizawa, 1988).

Table 1: Antioxidant and cytotoxic activities of the extracts and *nor*neolignans evaluated herein.

Samples	DPPH % inhibition	CC_{50} (µg/mL) Cell lines			
	at 66.7 µg/mL	GM07492A	HeLa	MO59J	MCF-7
MESC	18.47 ± 1.54[a,b]	270.8 ± 14.2[a]	-	-	-
MESF	2.40 ± 2.01[b]	164.7 ± 10.9[c]	-	-	-
1a	n. a.	1069.6 ± 35.1[a,c]	-	-	-
2a	4.93 ±0.99[b]	267.9 ± 1.9[a]	-	-	-
3	n. a.	19.10 ± 0.75[a,c]	1739.7 ± 327	> 2500	1411.7 ± 240.6
4	2.90 ± 1.83[b]	> 2500[a,c]	-	-	-
5	5.04 ± 2.19[b]	1897.7 ± 56.3[a,c]	-	-	-
6	n. a.	842.6 ± 31.2[a,c]	-	-	-
7	4.28 ± 0.92[b]	> 2500[a,c]	-	-	-
Gallic acid	92.41 ± 0.34[b]	-	-	-	-
Doxorubicin	-	7.8 ± 0.9[a,c]	21.9 ± 9.1	6.9 ± 2.1	5.4 ± 1.3

The values are the mean ± SD, n=3.
DPPH (2,2-diphenyl-1-picrylhydrazyl).
CC_{50} 50% cytotoxic concentration values for the different cell lines after 24-h treatment.
GM07492A (Human lung fibroblasts), MCF-7 (human breast adenocarcinoma), MO59J (human glioblastoma) and HeLa (human cervical adenocarcinoma).
[a] Significantly different from treatment with MESF ($p < 0.001$).
[b] Significantly different from treatment with gallic acid ($p < 0.001$).
[c] Significantly different from treatment with MESC ($p < 0.001$).



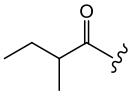
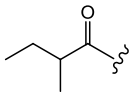
	R^1	R^2	R^3	R^4
1	H	OCH_3	CH_3	CH_3
1a	CH_3CO	OCH_3	CH_3	CH_3
2	H	OCH_3	$-CH_2-$	
2a	CH_3CO	OCH_3	$-CH_2-$	
3		H	$-CH_2-$	
4	Glu-Glu	OCH_3	$-CH_2-$	
5	H	H	$-CH_2-$	
6	H	H	CH_3	CH_3
7		OCH_3	$-CH_2-$	

Fig. 1: Chemical structures of *nor*neolignans.

Table 1 compiles the results achieved during the antioxidant assay of the isolated compounds. The evaluated *nor*neolignans were inactive as compared to gallic acid at 66.7 µg/mL. According to the literature (Hou *et al.*, 2003), the presence of compounds with stronger proton-donating capacity, such as phenolic compounds, is correlated to the DPPH scavenging activity. Based on our results, the compounds tested herein were almost inactive. The isolated compounds did not displayed free hydroxyl groups, so, the *nor*neolignans contributed to the extracts weak antioxidant activities.

To ascertain the toxicological properties of the assessed compounds, we conducted a 24-h XTT trial on normal human lung fibroblast cells (GM07492-A). Table 1 outlines the CC_{50} values. Treatment of the normal cells with compounds **1a**, **4**, **5**, **6** and **7** did not reduce cell viability significantly. These compounds were even less active than the MESC and MESF; e.g., compound **2a** yielded CC_{50} of 267.90 µg/mL, which showed it was less active than MESF.

Compound **3** had CC_{50} of 19.10 µg/mL, giving the most promising CC_{50} among the evaluated *nor*neolignas. To test the anticarcinogenic potential we used three cancer cells lines derived from different cancer types, human cervical adenocarcinoma (HeLa), human glioblastoma (MO59J), and human breast adenocarcinoma MCF-7 cells. For this reason, compound **3** was assayed on HeLa, MO59J, and MCF-7 cells, without effective reduction in cell viability.

The most active compounds (**2a** and **3**) have quite similar chemical structure: both bear an egonol core. The difference between their structures lies on the presence of acetate at R^1 in compound **2a**, whereas 2-methylbutanoate exists at R^1 and the methoxyl group at C-7 is absent in compound **3**. De Oliveira *et al.* (2016) had already evaluated the action of homoegonol (**1**) and egonol (**2**) on normal human cell (GM07492A), murine melanoma (B16F10), human cervical adenocarcinoma (HeLa), human hepatocellular liver carcinoma (HepG2), human breast adenocarcinoma (MCF-7), and human glioblastoma (MO59J). The authors established that a combination of compounds **1** and **2** and compound **2** alone exhibited CC_{50} values lower than 30 µg/mL for MCF-7 and for HepG2 at 72 h, respectively; selectivity indices were high.

Therefore, these authors suggested that the combination of compounds **1** and **2** as well as compound **2** alone should be a promising alternative for the development of anticancer drugs. In another study that used Kato III cells to evaluate drug cytotoxicity, egonol and demethoxy egonol presented *in vitro* cytotoxicity with IC_{50} values of 28.8 and 27.5 µg/ml, respectively (Yoshikawa *et al.*, 2001). These results again confirmed that the egonol core is a promising structural feature for anticancer drug research. According to Reiter *et al.* (2014), hybrids synthetized with egonol possess improved cytotoxic properties, thereby confirming the potential of the egonol structure in cancer research.

CONCLUSION

This is the first reported study on biological activities of fruits from *S. camporum* and *S. ferrugineus*. The present study provided evidence that the methanol extracts of *S. camporum* and *S. ferrugineus* fruits display weak antioxidant activity (18.47 %, and 2.40 % at 66.7 µg/mL), as compared with the gallic acid at this same concentration. These results stemmed from the fact that the structure of the *nor*neolignans present in these extracts do not bear a phenolic group. The extracts do not display cytotoxicity, either. The exception is compound **3**, which proved to be cytotoxic to normal cells. However, compound **3** is not cytotoxicity to evaluated cancer cells. The isolated *nor*neolignans could be used as starting material for synthesizing more cytotoxic compounds.

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