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Phenolic compounds and anticancer activity of commercial sugarcane cultivated in Brazil

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ABSTRACT

The cultivation of sugarcane hybrids (X *Saccharum officinarum* L.) is an important revenue source for the Brazilian economy. Herein it is reported the evaluation of the cytotoxic activity of mid-polarity sugarcane extracts against human cancer cell lines, as well as the isolation of steroids sitosterol, stigmasterol and campesterol, phenolic acids *p*-hydroxybenzoic, *p*-hydroxycinnamic, vanillic and ferulic acid, terpenoids α-tocopherol and β-carotene and a novel substance in sugarcane, the flavonoid aglycone tricin (5,7,4-trihydroxy-3,5-dimethoxyflavone). The presence of large amounts of phenolic acids and the flavonoid tricin may explain the cytostatic activity observed for the mid-polarity crude extract and filtrates.

Key words: anticancer activity, phenolic compounds, *Saccharum officinarum* L., sugarcane.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L., Poaceae), a grass native to Asia, is widely found in tropical regions and it is one of the most important cultures in Brazil, with an annual production of over 375 million tons (Conab 2013). It is widely used in sugar and alcohol production, yielding high quality at a low price for both products. Sugarcane is used to produce brown sugar bars (rapadura), molasses, rum (cachaça), in addition to its juice (garapa), which is largely consumed in Brazil.

Besides its great economic importance for food and fuel production, the literature reports medicinal properties for *S. officinarum* preparations, such as antidiabetic (Takahashi et al. 1985) and anti-mutagenicity (Yamashita et al. 1993) activities, as well as its use in osteoporosis treatment (Barros et al. 2007), usually associated with the juice prepared from the culms. The Ayurveda cites the use of this species to treat anemia, inflammations and ulcers (Chatterjee and Pakrashi 1995).

The most common phenolic compounds found in *S. officinarum* belong to the flavone class, such as naringenin, apigenin, tricin and luteolin derivatives (Williams et al. 1974, López-Lázaro 2009). These compounds are partly responsible for the sugarcane juice color and are acknowledged to have beneficial health effects associated with their high antioxidant potential and capacity to protect cells from degenerative processes and reduce the development of cancer and cardiovascular diseases.

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Phytochemical studies reported the presence and biological potential of these sugarcane compounds. A bioguided study of the juice from culms of *S. officinarum* led to isolation of the flavone tricin-7-*O*-β-(6''-methoxycinnamic)-glycoside, which has shown considerable antiproliferative and antioxidant potential (Almeida et al. 2007). Other studies have shown that extracts of sugarcane leaves could improve health quality, decreasing the incidence of diseases due to their antioxidant properties (Abbas et al. 2013). Other phytochemical studies reported the presence of flavonoid glycosides (Colombo et al. 2005, 2006a, b, Walford et al. 2009), wherever this class of natural products has been considered fundamental to human health.

In addition to the isolated flavonoids, other important bioactive compounds have been found in this species, such as hydroxamic acids, hydroxycinnamic acids, alpha hydroxy acids, aliphatic acids and polyamides (Loyd and Naidoo 1983). Alpha hydroxy acids are used in cosmetics for peeling (Ruciani 1997), in dermatology as a photoprotector and an anti-inflammatory (Perricone and Dinardo 1996). In the last years, the phenolic compounds of *S. officinarum* gained increasing attention regarding their antioxidant potential. The literature suggests the use of sugarcane as a dietary source of antioxidants, flavonoids like luteolin-8-*C*-(rhamnosyl glucoside), diosmentin-8-*C*-glucoside, vitexin, schaftoside, isoschaftoside and 4',5'-dimethyl-luteolin-8-*C*-glucoside, the major compounds responsible for this activity (Vila et al. 2008). Recent studies have reported this property (Ahmad et al. 2011, Duarte-Almeida et al. 2011).

The presence of anthocyanins in the methanolic crude extract from *S. officinarum* was recently reported (Pallavi et al. 2012). The same study demonstrated that the highly polar crude extract has cytotoxic potential against HT29 human colon cancer cells.

Although the presence of bioactive substances in highly polar sugarcane extracts is well known, little is known about mid-polarity extract compounds and their anticancer potential. Therefore, the antiproliferative activity of the ethyl acetate extract and fractions and the isolation of chemical constituents from the leaves of *S. officinarum* commercially cultivated in Brazil are reported herein.

MATERIALS AND METHODS

General Experimental Procedures

NMR spectra were obtained with a Varian spectrometer, model Mercury plus BB, operating at 300.06 MHz for ^1H and 75.5 MHz for ^{13}C. Chemical shifts were recorded in ppm with reference to internal tetramethylsilane (TMS $\delta = 0.0$ ppm). The solvents used were $CDCl_3$ and CD_3OD (Aldrich). Column chromatography (CC) was performed using silica gel 60 (Merck) or Sephadex LH-20 (Sigma). For thin layer chromatography (TLC), either silica gel 60 G or 60 GF254 (Merck) were employed. TLC spots were visualized by spraying an acetic acid/H_2SO_4/anysaldehyde solution (1:0.5:48.5 mL) followed by plate heating.

Gas chromatography coupled to mass spectrometry analyses were performed in a Focus GC (Thermo Finnigan) gas chromatograph coupled to a DSQ II (Thermo Finnigan) mass selective detector working with 70eV electron impact, fitted with a quadrupole type analyzer and an electron impact detector (FID). High-purity helium was used as mobile phase with a column flow of 1 mL min^{-1}. The analyses were performed with the injector working at 250 ºC and splitless injection. The capillary column used was DB-5 (30m x 0.25mm x 0.25µm) and the oven temperature program was from 100 °C to 290 ºC at 10 ºC min^{-1}. Sample aliquots were prepared from 1 mg of each compound dissolved in 1 mL of ethyl acetate (HPLC grade).

SUGARCANE SAMPLES, PREPARATION AND EXTRACT PURIFICATION

Saccharum officinarum samples were harvested from a commercial culture in the city of Munhoz de Mello, Paraná, Brazil. The material (4.36 kg) was crushed and exhaustively extracted with ethyl acetate at room temperature, yielding 72.00 g of crude extract. The extract was submitted to partition on a silica gel column eluted with hexane (7.64 g), ethyl acetate (33.75 g) and methanol (7.72 g).

ISOLATION OF CHEMICAL CONSTITUENTS

Part of the hexane partition (1.0 g) was submitted to silica gel column chromatography with hexane, chloroform and methanol in increasing polarity gradient. This procedure yielded β-carotene (**1**, 3.9 mg).

Silica gel column chromatography of the ethyl acetate partition (32.00 g) with hexane, hexane:dichloromethane, dichloromethane, dichloromethane:ethyl acetate, ethyl acetate, ethyl acetate:methanol and methanol in increasing polarity gradient yielded 548 fractions. The fractions eluted with CH_2Cl_2:AcOEt (85:15) were combined and washed with ethyl acetate, leading to the isolation of flavonoid tricin (**8**, 55.0 mg). The fractions eluted with CH_2Cl_2:AcOEt (3:2) were combined, washed with cold propanone and yielded compound sitosterol glucoside (**9**, 104.2 mg).

Similarly, part of the fractions eluted with CH_2Cl_2:AcOEt (9:1) were combined and purified in Sephadex LH 20 with methanol as eluent. This procedure led to the isolation of a mixture of *p*-hydroxycinnamic and *p*-hydroxybenzoic acids (**10** and 11, 33.5 mg). The fractions eluted with 100% dichloromethane were combined and washed with ethyl acetate and methanol and yielded a mixture of steroids sitosterol, stigmasterol and campesterol (**2**-**4**, 30.0 mg). After, the remaining dough of fractions combined (2.08 g) was submitted to silica gel column chromatography with hexane, chloroform and methanol in increasing polarity gradient yielding the compound α-tocopherol (**5**, 3.6 mg). The fractions eluted with $HCCl_3$:MeOH 20% were combined and purified in silica gel column chromatography with hexane, ethyl acetate and methanol in increasing polarity gradient. This procedure yielded compounds vanillic acid (**6**, 6.0 mg) and ferulic acid (**7**, 5.0 mg).

SPECTRAL DATA OF THE COMPOUNDS 1-11 OBTAINED IN THIS WORK

*Sitosterol (**2**)*. GC-MS (EI, 70 eV) *m/z*: 414. 31 (M^+ 1), 55.00 (100), 69.01 (93.3), 83.05 (79.8), 80.99 (76.0). ^{1}H NMR (300.06 MHz, $CDCl_3$). δ 3.47 (1H, m, H-3), 5.36 (1H, d; 5.1, H-6), 0.68 (3H, s, H-18), 1.01 (3H, s, H-19), 0.93 (3H, s, H-21), 0.84 (3H, s, H-26), 0.81 (3H, s, H-27), 0.87 (3H, s, H-29). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 37.4 (C-1), 31.8 (C-2), 72.0 (C-3), 42.5 (C-4), 140.9 (C-5), 121.9 (C-6), 31.8 (C-7), 32.1 (C-8), 50.3 (C-9), 36.3 (C-10), 21.2 (C-11), 39.8 (C-12), 42.5 (C-13), 56.9 (C-14), 24.5 (C-15), 28.4 (C-16), 56.2 (C-17), 12.0 (C-18), 19.6 (C-19), 36.1 (C-20), 19.6 (C-21), 33.9 (C-22), 39.2 (C-23), 46.0 (C-24), 29.1 (C-25), 18.9 (C-26), 19.6 (C-27), 23.2 (C-28), 12.2 ppm (C-29).

Stigmasterol (3). GC-MS (EI, 70 eV) *m/z*: 412.26 (M^+ 2), 43.02 (100), 105.01 (84.9), 95.05 (84.6), 107.03 (93.59). ^{1}H NMR (300.06 MHz, $CDCl_3$). δ 3.47 (1H, m, H-3), 5.36 (1H, d; 5.1, H-6), 4.97 (1H, dd; 8.4, H-22), 5.19 (1H, dd; 8.4, H-23), 0.68 (3H, s, H-18), 1.01 (3H, s, H-19), 0.93 (3H, s, H-21), 0.84 (3H, s, H-26), 0.81 (3H, s, H-27), 0.87 (3H, s, H-29). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 37.4 (C-1), 31.8 (C-2), 72.0 (C-3), 42.5 (C-4), 140.9 (C-5), 121.9 (C-6), 31.8 (C-7), 32.1 (C-8), 50.3 (C-9), 36.3 (C-10), 21.2 (C-11), 39.8 (C-12), 42.5 (C-13), 56.9 (C-14), 24.5 (C-15), 28.4 (C-16), 56.1 (C-17), 12.0 (C-18), 19.6 (C-19), 40.7 (C-20), 21.4 (C-21), 138.5 (C-22), 129.4 (C-23), 51.4 (C-24), 31.8 (C-25), 19.2 (C-26), 19.2 (C-27), 25.6 (C-28), 12.4 ppm (C-29).

*Campesterol (**4**)*. GC-MS (EI, 70 eV) *m/z*: 400.28 (M^+ 71.7), 43.97 (100), 43.03 (60.9), 145.07 (45.0), 95.06 (39.9). ^{1}H NMR (300.06 MHz, $CDCl_3$). δ 3.47 (1H, m, H-3), 5.36 (1H, d; 5.1, H-6), 0.68 (3H, s, H-18), 1.01 (3H, s, H-19), 0.93 (3H, s, H-21), 0.84 (3H, s, H-26), 0.81 (3H, s, H-27), 0.78 (3H, s, H-28). ^{13}C NMR (75.5 MHz, $CDCl_3$). δ 37.4 (C-1), 31.8 (C-2), 72.0 (C-3), 42.5 (C-4), 140.9 (C-5), 121.9 (C-6), 31.8 (C-7), 32.1 (C-8), 50.3 (C-9), 36.3 (C-10), 21.2 (C-11), 39.9 (C-12), 42.5 (C-13), 56.9 (C-14), 24.5 (C-15), 28.4 (C-16), 56.2 (C-17), 12.0 (C-18), 19.6 (C-19), 36.1 (C-20), 18.9 (C-21), 33.9 (C-22), 29.1 (C-23), 56.1 (C-24), 29.6 (C-25), 18.9 (C-26), 19.6 (C-27), 18.9 ppm (C-28).

*Tricin (**8**)*. GC-MS (EI, 70 eV) *m/z*: 329.91 (M^+ 100), 152.90 (11.4), 43.98 (9.8), 151.04 (8.45), 206.92 (7.44). ^{1}H NMR (300.06 MHz, CD_3OD/$CDCl_3$). δ 7.19 (s, H-2' and 6'), 6.60 (s, H-3), 6.23 (d; 2.1, H-6), 6.46 (d; 2.1, H-8), 3.98 ppm (s, 3'-OCH_3 and 5'-OCH_3). ^{13}C NMR (75.45 MHz, CD_3OD/$CDCl_3$). δ 165.3 (C2), 104.9 (C-3), 183.1 (C-4), 162.4 (C-5), 99.0 (C-6), 164.9 (C-7), 94.8 (C-8), 158.2 (C-9), 104.8 (C-10), 104.4 (C-2'), 148.7 (C-3'), 140.2 (C-4'), 148.7 (C-5'), 104.4 (C-6'), 56.0 ppm (3'-OCH_3 and 5'-OCH_3).

*p-hydroxycinnamic acid (**10**)*. GC-MS (EI, 70 eV) *m/z*: 163.98 (M^+ 100), 163.99 (41.5), 162.97 (39.8), 118.99 (25.5), 117.97 (23.3). ^{1}H NMR (300.06 MHz, CD_3OD/$CDCl_3$). δ 7.45 (2H, dt; *J*= 8.4 e 4.8 Hz, H-2 e H-6) 6.81 (2H, dt; *J*= 8.4 e 4.8 Hz, H-3 e H-5), 6.30 (1H, d; *J*= 15 Hz; H-8), 7.62 ppm (1H, d; *J*= 15 Hz; H-7). ^{13}C NMR (75.5 MHz, CD_3OD/$CDCl_3$). δ 127.2 (C-1), 131.0 (C-2, C-6), 116.0 (C-3, C-5), 161.1 (C-4), 146.6 (C-7), 115.6 (C-8), 170.0 ppm (C=O).

*p-hydroxybenzoic acid (**11**)*. GC-MS (EI, 70 eV) *m/z*: 137.94 (M^+ 5.1), 120.94 (100), 137.94 (70.3), 64.98 (16.4), 121.97 (7.9). NMR ^{1}H (300.06 MHz, CD_3OD/$CDCl_3$). 7.90 (2H, dt; *J*= 8.4 e 4.8 Hz, H-2 e H-6), 6.81 (2H, dt; *J*= 8.4 e 4.8 Hz, H-3 e H-5). ^{13}C NMR (75.45 MHz, CD_3OD/$CDCl_3$). δ 131.0 (C-1), 132.9 (C-2, C-6), 116.7 (C-3, C-5), 163.3 (C-4), 171.0 ppm (C=O).

*Ferulic acid (**7**)*. GC-MS (EI, 70 eV) *m/z*: 193.97 (M^+ 100), 43.97 (20.6), 178.94 (19.4), 132.94 (17.5), 192.98 (11.3). ^{1}H NMR (300.06 MHz, CD_3OD) δ 7.17 (1H, d, 2.1 Hz, H-2), 6.80 (1H, d; 8.4 Hz, H-5) 7.06 (1H, dd; 8.4 e 2.1 Hz, H-6), 7.58 (1H, d, 15.9 Hz, H-7), 6.33 (1H, d, 15.9 Hz), 3.87 (OCH_3). ^{13}C NMR (75.45 Hz, CD_3OD), δ 128.6 (C-1), 111.5 (C-2), 151.9 (C-3), 149.3 (C-4), 116.4 (C-5), 123.0 (C-6), 146.2 (C-7), 116.9 (C-8), 56.4 (OCH_3), 171.0 ppm (C-9).

*Vanillic acid (**6**)*. GC-MS (EI 70 eV) *m/z* 167.95 (M^+ 100), 152.98 (80.1), 96.92 (25.8), 124.94 (21.6), 150.98 (18.3). ^{1}H NMR (300.06 MHz, CD_3OD) δ 7.54 (2H, m, H-2 e H-6), 6.84 (1H, d; 8.7 Hz, H-5), 3.88 ppm (3H, s, OCH_3). ^{13}C NMR (75.45 Hz, CD_3OD) δ 128.4 (C-1), 113.7 (C-2), 151.9 (C-3), 152.7 (C-4), 115.8 (C-5), 125.5 (C-6), 170.0 (C-7), 56.4 ppm (OCH_3).

α-tocopherol (***5***). GC-MS (EI, 70 eV) *m/z*: 430.28 (M^+ 70.8), 165.03 (100), 164.03 (34.4), 166.04 (12.2), 205.04 (11.4). ^{1}H NMR (300.06 MHz, $CDCl_3$). δ 2.61 (2H, t; 6.9, H-4), 1.78 (2H, m, H-3), 2.17 (3H, s, H-8b), 2.12 (3H, s, H-7a), 1.59 (3H, s, H-5a), 1.26 (3H, s, H-2a), 0.88 (3H, s, H-4'a), 0.87 (3H, s, H-8'a), 0.85 (6H, s, H-12'a and H-13'a) ^{13}C NMR (75.45 MHz, $CDCl_3$) δ 145.7 (C-8a), 122.8 (C-8), 118.7 (C-7), 11.5 (C-7a), 144.7 (C-6), 117.6 (C-5), 12.0 (C-5a), 121.9 (C-4a), 24.7 (C-4), 31.7 (C-3), 74.7 (C-2), 39.6 (C-1'), 24.7 (C-2'), 37.3 (C-3', 4',5',7', 8', 9'), 25.0 (C-6'), 24.6 (C-10'), 39.9 (C-11'), 28.2 (C-12'), 22.9 (C-12'a, 13'), 19.9 (C-8'a, 4'a), 24.0 (C-2a), 12.4 ppm (C-8b).

*Sitosterol glucoside (**9**)*. GC-MS (EI, 70 eV) *m/z*: 414.31 (M^+ 0), 43.95 (100), 206.93 (17.5), 43.01 (15.1), 164.96 (6.4). ^{1}H NMR (300.06 MHz, CD_3OD/$CDCl_3$). δ 3.37 (1H, m, H-3), 5.32 (1H, d; 5.1, H-6), 0.67 (3H, s, H-18), 1.03 (3H, s, H-19), 0.92 (3H, s, H-21), 0.84 (3H, s, H-26), 0.83 (3H, s, H-27), 0.89 (3H, s, H-29), 4.40 (1H, d; 8.1, H-1'),

3.53 (1H, m, H-2'), 3.46 (1H, m, H-3'), 3.35 (1H, m, H-4'), 3.70 (1H, m, H-5'), 3.54 (1H, m, H-6'a), 4.06 (1H, m, H-6'b). ^{13}C NMR (75.5 MHz, CD_3OD) δ 37.5 (C-1), 32.7 (C-2), 79.7 (C-3), 43.0 (C-4), 141.3 (C-5), 122.7 (C-6), 32.7 (C-7), 32.8 (C-8), 51.1 (C-9), 36.9 (C-10), 21.8 (C-11), 39.4 (C-12), 43.0 (C-13), 56.8 (C-14), 25.0 (C-15), 29.0 (C-16), 57.6 (C-17), 12.3 (C-18), 19.8 (C-19), 36.9 (C-20), 20.5 (C-21), 34.7 (C-22), 40.6 (C-23), 46.7 (C-24), 29.0 (C-25), 19.8 (C-26), 21.8 (C-27), 23.8 (C-28), 12.7 (C-29), 102.0 (C-1'), 74.5 (C-2'), 78.7 (C-3'), 71.1 (C-4'), 77.1 (C-5'), 62.5 ppm (C-6').

*β-carotene (**1**)*. GC-MS (EI, 70 eV) *m/z*: 536.97 (M^+ 0), 43.99 (100), 43.04 (18.1), 55.02 (13.9), 57.04 (11.9). ^{1}H NMR (300.06 MHz, $CDCl_3$). δ 2.02 (4H, m, H-4 and H-4'), 1.64 (4H, m, H-3 and H-3'), 1.46 (4H, m, H-2 and H-2'), 1.03 (6H, s, H-16 and H-16'), 1.04 (6H, s, H-17 and H-17'), 1.72 (6H, s, H-18 and H-18'), 1.97 (6H, s, H-19 and H-19'), 2.05 (6H, s, H-20 and H-20'). ^{13}C NMR (75.45 MHz, $CDCl_3$) δ 34.5 (C-1/1'), 39.9 (C-2/2'), 19.5 (C-3/3'), 33.3 (C-4/4'), 129.6 (C-5/5'), 137.9 (C-6/6'), 126.6 (C-7/7'), 137.5 (C-8/8'), 136.7 (C-9/9'), 131.1 (C-10/10'), 125.2 (C-11/11'), 137.2 (C-12/12'), 136.7 (C-13/13'), 132.6 (C-14/14'), 129.6 (C-15/15'), 29.2 (C-16/16', 17/17'), 22.7 (C-18/18'), 12.9 (C-19/19'), 12.9 ppm (C-20/20').

In vitro ANTICANCER ASSAY

The *in vitro* anticancer assays of the ethyl acetate extract and its hexane, ethyl acetate and methanol partitions and pure flavonoid tricin were performed as described in the literature (Monks et al. 1991). Eight human tumor cell lines [U251 (glioma), MCF-7 (breast), NCI-ADR/RES (multiple drug resistant ovary cells), 786-0 (kidney), NCI-H460 (lung, non-small cells), PC-3 (prostate), OVCAR-03 (ovary) and colon (HT29)] were kindly provided by Frederick MA, National Cancer Institute/USA. HaCat (human keratinocytes) cell line was kindly donated by Dr. Ricardo Della Coletta (FOP, UNICAMP). Stock and experimental cultures were grown in media containing 5 mL RPMI 1640 (GIBCO BRL) supplemented with 5 % fetal bovine serum (GIBCO BRL). A penicillin:streptomycin mixture (1000 µg mL^{-1} : 1000 µg mL^{-1}, 1 mL L^{-1} RPMI) was added to the experimental cultures. Cells plated to 96-well plates (100 µL cells $well^{-1}$) were exposed to different sample concentrations in DMSO/RPMI (0.25, 2.5, 25 and 250 µg mL^{-1}) at 37 °C under 5% CO_2 in air for 48 h. The final DMSO concentration did not affect cell viability. The cells were fixed with 50% trichloroacetic acid before (T_0 plate) and after (T_1 plates) sample addition. Cell proliferation was determined by spectrophotometric quantification (540 nm) of cell protein content using the sulforhodamine B assay. Using the concentration-response curve for each cell line, the GI_{50} (concentration resulting in growth inhibition of 50%) was determined through non-linear regression analysis in software ORIGIN 8.0 (OriginLab Corporation) (Shoemaker 2006).

RESULTS AND DISCUSSION

β-carotene (**1**), steroids sitosterol (**2**), stigmasterol (**3**) and campesterol (**4**), α-tocopherol (**5**), vanillic (**6**) and ferulic acids (**7**), tricin (**8**), sitosterol glucoside (**9**) and *p*-hydroxycinnamic (**10**) and *p*-hydroxybenzoic acids (**11**) (Figure 1) were isolated from the ethyl acetate extract and identified by interpretation of ^{1}H, ^{13}C, DEPT and COSY NMR spectral data and comparison with literature data (Miranda et al. 2012, Goulart et al. 1993, Yu et al. 2006, Sajjadi et al. 2012, Chung et al. 2005, Lee et al. 2007, Silva et al. 2001, Scott 1972).

The molecular formula of compound **8**, $C_{17}H_{14}O_7$, was established by GC-MS analysis [*m/z* 329.91]. Its ^{1}H NMR spectrum presented signals characteristic of a substance belonging to the flavonoid class, of which the following stand out the singlet at δ_H 7.19 relative to hydrogens H-2' and H-6' of ring B and doublets at δ_H 6.46 (*J*= 2.1 Hz)

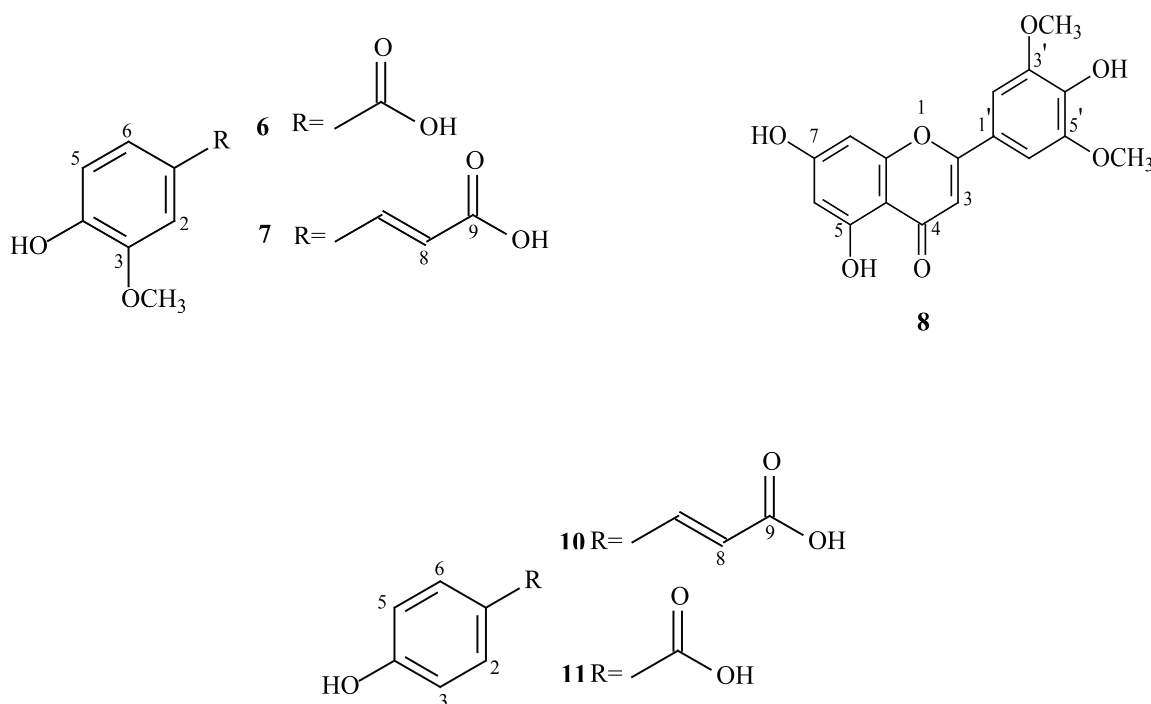

Figure 1 - Phenolic compounds isolated from sugarcane.

and 6.23 (J= 2.1 Hz), corresponding to hydrogens H-8 and H-6 of ring A, and a singlet at δ_H 6.60 attributed to hydrogen H-3. The ^{13}C NMR spectrum showed resonances at δ_C 158.6, 94.8, 164.9, 99.6, 162.2 and 104.9, attributed to carbons C-9, C-8, C-7, C-6, C-5 and C-10 of ring A, respectively. Furthermore, a signal at δ_C 183.1, characteristic of the carbonyl 1,4-pirone system, and signals in the δ_C 122.0-165.3 region, attributed to ring B carbons, were observed. The signals at δ_C 104.9 and 165.3, corresponding to carbons C-3 and C-4 of ring C, are typical of flavone aglycone. These data were compared with data in the literature (Khadem and Marles 2010) and were consistent for tricin.

The ethyl acetate extract from young leaves and culms of *S. officinarum* and the hexane, ethyl acetate and methanol partitions from the ethyl acetate extract were tested at concentrations ranging from 0.25 to 250 µg/mL against 08 different human tumor cell lines [glioma (U521), breast (MCF-7), resistant ovary (NCI/ADR-RES), kidney (786-0), lung (NCI-H460), prostate (PC-3), ovary (OVCAR-3) and colon (HT29)] and non-tumoral human keratinocyte (HaCat) cell lines using a colorimetric method for growth inhibition evaluation (Table I). Doxorubicin was used as a positive control.

Human cell lines: A = U251 (glioma, SNC); B = MCF-7 (breast); C = NCI-ADR/RES (multiple drug resistant ovary cells); D = 786-0 (kidney); E = NCI-H460 (lung); F = PC-3 (prostate); G = OVCAR-3 (ovary); H = HT29 (colon); Q = HaCat (normal human keratinocytes). *Doxorubicin: reference chemotherapy; GI_{50}: Concentration necessary for 50% cell growth inhibition.

In general, the ethyl acetate extract showed cytostatic activity in concentrations ranging from 25.8 to 61.8 µg/mL. Similar activity was noticed for the ethyl acetate partition, suggesting that the compounds responsible for this biological activity were concentrated in this fraction. Corroborating these results, the hexanic and methanolic fractions

TABLE I
GI_{50} (µg/mL) for the *S. officinarum* crude ethyl acetate extract and partitions for cancer cell lines.

	A	B	C	D	E	F	G	H	Q
Doxorubicin *	< 0.025	< 0.025	0.025	0.20	< 0.025	0.082	0.28	0.12	0.025
EtAc extract	29.0	25.8	25.4	31.8	28.7	47.8	57.9	61.8	26.1
Hexane part.	> 250	164.0	162.5	> 250	> 250	> 250	> 250	> 250	> 250
EtAc part.	29.1	26.1	5.9	65.4	51.7	30.3	52.4	35.0	34.2
Methanol part.	> 250	67.2	> 250	> 250	> 250	> 250	> 250	> 250	> 250
pure tricin	> 250	> 250	70.3	> 250	127.7	> 250	41.1	> 250	69.6

showed a comparatively lower activity, being inactive (GI_{50} > 250 µg/mL) against almost all cell lines (Table I). Thus, fractionation of the ethyl acetate fraction was prioritized.

Of the compounds isolated from the ethyl acetate fraction, flavonoid tricin was evaluated against the same cell line panel. Tricin showed cytostatic activity against two ovarian cancer cell lines, OVCAR-3 (GI_{50} = 41.1 µg/mL) and NCI-ADR/RES (GI_{50} = 70.3 µg/mL). As observed for doxorubicin, tricin also inhibited cell growth of non tumoral human keratinocytes (HaCat, GI_{50} = 69.6 µg/mL). Therefore, this result partly explains the biological activity observed for the ethyl acetate crude extract and the ethyl acetate fraction.

Based on literature results (Kampa et al. 2003, Lee 2005, Pugazhendhi et al. 2005) for the other phenolic compounds isolated from the bioactive ethyl acetate fraction (*p*-hydroxybenzoic, *p*-hydroxycinnamic, vanillic and ferulic acid), it is possible to postulate the involvement of these compounds in the cytostatic activity observed for the ethyl acetate crude extract and the ethyl acetate fraction. (Cai et al. 2004) evaluated tricin in a xenographic breast tumor model and observed that treating animals with tricin (0.2% w/w in diet) for 7 days before tumor cell (MDA-MB-468) implantation did not reduce tumor growth, but tricin treatment (72 h, 11µM) before MDA-MB-468 tumor cell implantation in nude mice resulted in smaller tumors when compared to untreated cells. Besides, extracts containing high tricin concentrations are known to inhibit human colon and breast cancer cell proliferation (Hudson et al. 2000). There is also evidence that tricin has chemopreventive activity, such as observed in intestinal carcinogenesis models in mice (Cai et al. 2005).

The phytochemical study of the leaves and culms of *Saccharum officinarum* led to the isolation of eleven compounds belonging to different classes. Their structures were identified based on GC-MS and NMR methods. The tricin showed significant anticancer activity in the *in vitro* anticancer assay. Therefore, the present isolation of tricin corroborates anticancer studies, pointing to the optimization of the production of tricin from this new source for important pharmacological studies. Sugarcane may also be an important source of tricin derivatives which may be used to produce the bioactive aglycone by hydrolysis.

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