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Original Article

New flavone and other compounds from *Tephrosia egregia*: assessing the cytotoxic effect on human tumor cell lines

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ABSTRACT

The organic extracts from stems, roots and leaves of *Tephrosia egregia* Sandwith, Fabaceae, provided a new flavone, 5-hydroxy-8-(1″,2″-epoxy-3″-hydroxy-3″-methylbutyl)-7-methoxyflavone (**1**), in addition to eleven known compounds: pongaflavone (**2**), praecansone B (**3**), 12a-hydroxyrotenone (**4**), praecansone A, 2′,6′-dimethoxy-4′,5′-(2″,2″-dimethyl)-pyranochalcone, pongachalcone, maackiain, β-sistosterol and its glucoside, *p*-cumaric acid and cinnamic acid. The structures of all compounds were established on the basis of spectroscopic methods, mainly 1D and 2D NMR and HRESIMS, involving comparison with literature data. Cytotoxicity of compounds **1–4** was evaluated against AGP-01 (cancerous ascitic fluid), HCT-116 (colon adenocarcinoma), HL-60 (leukemia), PC-3 (prostate carcinoma), SF-295 (glioblastoma) and SKMEL 28 (melanoma) cell lines.

Introduction

Tephrosia egregia Sandwith, Fabaceae, popularly known as "anil-bravo" (Moreira and Bragança, 2011), is a perennial herbaceous plant that occurs mainly in sub-saline soils of tropical and sub-tropical areas (Bolland, 1947). Some *Tephrosia* species are used as natural insecticide and possess several biological activities such as fish-poisoning, estrogenic, antitumor, antimicrobial, antiprotozoal, antifeedant, antioxidant, larvicidal and anti-inflammatory (Arriaga et al., 2005; Ribeiro et al., 2006; Vasconcelos et al., 2009; Juma et al., 2011; Do Val et al., 2014; Chen et al., 2014). In previous investigations of *T. egregia* we reported the chemical composition of its essential oil and the occurrence of flavonoids in the roots (Arriaga et al., 2005, 2009).

Further examination of this plant led to the isolation and characterization of a new compound, the 5-hydroxy-8-(1″,2″-epoxy-3″-hydroxy-3″-methylbutyl)-7-methoxyflavone (**1**), along with the known flavonoids pongaflavone (**2**), praecansone B (**3**) (Vasconcelos et al., 2009), 12a-hydroxyrotenone (**4**) (YouZhi et al., 2011), praecansone A (**5**) (Taurus et al., 2002), 2′,6′-dimethoxy-4′,5′-(2″,2″-dimethyl)-pyranochalcone (**6**) (Ganapaty et al., 2008), pongachalcone (**7**) (Andrei et al., 2000), maackiain (**8**) (Sato et al., 2007) from its roots and stems. Furthermore, β-sistosterol (De-Eknambul and Potduang, 2003) and its glucoside (Korde et al., 1996), as well as, two phenyl propanoids, the *p*-cumaric acid (Souza-Filho et al., 2009) and cinnamic acid (Prachayasittikul et al., 2009) were obtained from its leaves. In addition, we evaluated the cytotoxic activity (Mosmann, 1983) against AGP-01 (cancerous ascitic fluid), HCT-116 (colon adenocarcinoma), HL-60 (leukemia), PC-3 (prostate carcinoma), SF-295 (glioblastoma) and SKMEL 28 (melanoma) cell lines to the compounds **1–4**.

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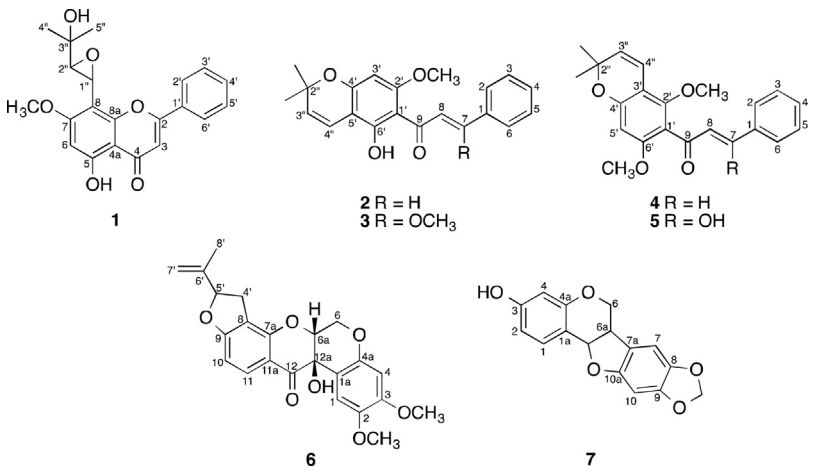

Material and methods

General experimental procedures

The spectra of ^{1}H and ^{13}C NMR, 1D and 2D, were obtained in Bruker Avance DRX-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) and DPX-300 (300 MHz for ^{1}H and 75 MHz for ^{13}C) spectrometers. All spectra were run using deuterated solvent $CDCl_3$, C_5D_5N and CD_3OD. The infrared spectra were obtained using a Perkin-Elmer, Spectrum 100 FTIR spectrometer, equipped with UATR accessory. Low resolution mass spectra (electron impact mode 70 eV) were obtained in a Shimadzu GCMS-QP 2010 by direct injection. The high resolution mass spectra were recorded on an Acquity UPLC chromatograph m coupled to Quadrupole/Time-of-Flight system (UPLC/Qtof MSE spectrometer) and in a Shimadzu LCMS-IT-TOF. The melting point was detected in Marconi MA-381 apparatus. Optical rotations were measured in $CHCl_3$ and $CHCl_3$/MeOH solutions on a JASCO model P-2000 polarimeter equipped with lamp of I-W and filter of 589 nm. For preparative thin layer chromatography (TLC), silica gel 60 F_{254}-coated plates (20 × 20 cm × 0.5 mm) were used for monitoring fractions pure. For column chromatography, silica gel 60 (Ø 0.04–0.020 mm; Merk) and Sephadex LH-20 were employed. The HPLC separations were performed in a Shimadzu LC-20AT chromatograph, equipped with PDA detector and Phenomenex RP 18 (250 × 10 mm, 5 μm) column, using a mixture of MeOH and H_2O as eluent.

Plant material

Tephrosia egregia Sandwith, Fabaceae, was collected in September, 2014 in Caucaia, Ceará state, Brazil. A voucher specimen (#55945) was deposited at the Prisco Bezerra Herbarium (UFC-CE), Brazil.

Extraction and isolation

The air-dried and powdered stems of *T. egregia* (1.52 kg) were extracted with EtOH at room temperature and furnished 62 g of extract. The crude extract was subjected to partition process with hexane, CH_2Cl_2 and EtOAc, yielding the corresponding fractions after solvent distillation [hexane (5.2 g), CH_2Cl_2 (4 g) and EtOAc (637.2 mg)]. The CH_2Cl_2 (4 g) fraction was chromatographed on silica gel column successively eluted with gradient mixture of hexane, CH_2Cl_2 and EtOAc. The fraction eluted with EtOAc (251.5 mg) was purified by reversed-phase HPLC using an isocratic method composed of MeOH/H_2O (60:40), leading to the isolation of compound **1** (5.2 mg).

The air-dried and powdered roots (2.5 kg) of *T. egregia* were extracted with EtOH at room temperature to furnish 56.8 g of crude extract. Next, this extract was subjected to silica gel column chromatography using hexane, hexane/CH_2Cl_2 (50:50), CH_2Cl_2, CH_2Cl_2/EtOAc (50:50), EtOAc and MeOH as eluents, resulting in the fractions F1-F6, respectively. Fraction F1 (20.5 g), was chromatographed on silica gel column and eluted with hexane, CH_2Cl_2 and CH_2Cl_2:MeOH (90:10; 80:20 and 50:50). The fraction eluted with hexane furnished β-sitosterol. The fraction eluted with CH_2Cl_2 (264.5 mg) was purified over Sephadex LH-20 and provided the compound **7** (18.5 mg). Fraction F2 (8.2 g) was chromatographed on a silica gel column with mixtures of hexane, CH_2Cl_2 and EtOAc with increasing polarity. The hexane:EtOAc (90:10) fraction provided the compounds **8** (7.6 mg) and **5** (10.0 mg).

Air-dried and powdered roots (500 g) of *T. egregia* was extracted with EtOH in a Soxhlet apparatus and furnished 57.8 g of crude extract. An aliquot of this latter one (6.8 g) was fractionated on a silica gel column using mixtures of hexane, CH_2Cl_2, EtOAc and MeOH with increasing polarity. The fraction eluted with hexane:EtOAc (1:1) (912.5 mg) was subjected to silica gel column chromatography with hexane: CH_2Cl_2 (90:10; 80:20; 65:35; 50:50; 30:70; 10:90) as eluents. After TLC analysis of the fractions, they were grouped into five sub-fractions. The sub-fraction 2, by elution with a mixture of hexane/CH_2Cl_2 (90:10) was purified by preparative thin layer chromatography to afford (19.3 mg) and **3** (7.5 mg). The sub-fraction eluted with hexane:CH_2Cl_2 (80:20) (149.5 mg) was purified by HPLC using MeOH:H_2O (75:25) as mobile phase and yielded compound **6** (7 mg) and **2** (22.7 mg). The fraction CH_2Cl_2 (636.2 mg) was subjected to silica gel column chromatography by elution with CH_2Cl_2:EtOAc (90:10; 80:20; 60:40; 50:50; 30:70) and MeOH. The fraction eluted with CH_2Cl_2:EtOAc (60:40) was purified over Sephadex LH-20 eluted with CH_2Cl_2:MeOH (50:50) and yielded compound **4** (7 mg).

The aqueous extract obtained by hydrodistillation of *T. egregia* leaves (500 g) was concentrated under reduced pressure and exhaustively extracted with EtOH. The organic extract (1.8 g) was chromatographed over Sephadex LH-20 column, by elution with MeOH, and provided 12 fractions. Further purification of the fraction 6 (220.0 mg) by chromatography on silica column yielded the compounds glucoside (7 mg), *p*-cumaric acid (8.6 mg) and cinnamic acid (10.1 mg) eluted with hexane: EtOAc (50:50), hexane:EtOAc (30:70) and EtOAc:MeOH (80:20), respectively.

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound **1**, including results obtained by HMQC (^{1}JCH) and HMBC (^{2}JCH and ^{3}JCH) spectra, in C_5D_5N as solvent. The chemical shifts in δ (ppm) and coupling constants (J, in parenthesis) in Hz[a].

	HMQC		HMBC	
	δ_C	δ_H	H ($^{2}J_{C-H}$)	H ($^{3}J_{C-H}$)
2	160.7, C	–	H-3	H-2′/H-6′
3	109.8, CH	7.02 (1H; s)	–	–
4	177.1, C	–	H-3	H-6 ($^{4}J_{CH}$)
4a	109.8, C	–	–	H-6
5	158.9, C	–	H-6	–
6	97.8, CH	6.57 (1H; s)	–	–
7	161.4, C	–	H-6	MeO-7
8	106.1, C	–	H-1″	H-6
8a	159.8, C	–	–	H-1″
1′	131.8, C	–	H-2″; 3H-4″; 3H-5″	H-1″
2′/6′	126.9, CH	8.16 (2H; d)	–	H-4′
3′/6′	129.7, CH	7.40 (2H; t)	–	–
4′	131.7, CH	7.44 (1H; t)	–	H-2′/H-6′
1″	62.7, CH	5.55 (1H; br; 4.2)	H-2″	–
2″	73.3, CH	4.22 (1H; br; 4.2)	H-1″	3H-4″; 3H-5″
3″	80.5, C	–	H-2″; 3H-4″; 3H-5″	H-1″
MeO-7	56.6, CH_3	3.85 (3H; s)	–	–
4″	22.3, CH_3	1.89 (3H; s)	–	H-2″
5″	27.7, CH_3	1.74 (3H; s)	–	H-2″

[a] Number of hydrogens bound to carbon atoms deduced by comparative analysis of {^{1}H}- and DEPT-^{13}C NMR spectra. Chemical shifts and coupling constants (J) obtained from 1D ^{1}H NMR spectrum. Superimposed ^{1}H signals are described without multiplicity and chemical shifts deduced by HMQC, HMBC and ^{1}H–^{1}H-COSY spectra.

Cytotoxicity activity

Cell lines HCT-116 (colon adenocarcinoma), HL-60 (leukemia), PC-3 (prostate carcinoma), SF-295 (glioblastoma) and SKMEL 28 (melanoma) were kindly provided by the National Cancer Institute (Bethesda, MD, USA), while the cell line AGP-01 (cancerous ascitic fluid) was obtained from a malignant ascites due to a primary gastric tumor (Leal et al., 2009). The cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin at 37 °C with 5% CO_2. Cytotoxicity was evaluated using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) (Sigma–Aldrich Co., St. Louis, MO/USA) reduction assay (Mosmann, 1983). For all experiments, cells were seeded in 96 well plates (5×10^3 cells/well) and compounds **1–4** (0.19–25 μg/ml) were added to each well and incubated for 72 h. After 69 h of incubation, the supernatant was replaced by fresh medium containing MTT (0.5 mg/ml). Three hours later, the MTT formazan product was dissolved in 150 μl of DMSO and absorbance was measured at 595 nm (DTX 880 Multimode Detector, Beckman Coulter, Inc. Fullerton, CA, USA).

Results and discussion

Compound **1** was isolated as a white solid, with mp: 260 °C (decomp.) and $[\alpha]^{25}_D = +35.90°$ ($c = 0.1$; CH_2Cl_2/MeOH 1:1). Its molecular formula $C_{21}H_{20}O_6$ was established by the analysis of the HRESIMS, which exhibited a quasimolecular ion peak ($[M+H]^+$) at m/z 369.1336 (calculated: m/z 369.1338). Its IR spectrum showed chromone carbonyl absorption at ν_{max} 1643 cm^{-1} and epoxy group absorption at ν_{max} 1207 cm^{-1}. The ^{13}C NMR spectrum of the compound showed one absorption at δ_C 177.2 due to a chromone carbonyl carbon with a chelated hydroxyl group (Supplementary material).

The ^{1}H NMR spectrum of **1** revelead the presence of signals attributed to an unsubstitute phenyl ring (δ_H 7.36–8.15; 5H; m) and a singlet to one methoxyl group at δ_H 3.83. The signal corresponding to H-3 hydrogen appeared as a singlet at δ_H 7.02. The spectrum also showed two additional at δ_H 4.22 (br s, H-2″ correlated in the HSQC spectrum with the carbon signal at δ_C 73.3, OCH_2″) and δ_H 5.55 (br s, H-1″ correlated in the HSQC spectrum with the carbon signal at δ_C 62.7, OCH-1″ relatively protected by γ-effects of two methyl groups CH_3-4″ and CH_3-5″ together with a hydroxyl group), each integrating for one hydrogen and revealing correlation in the COSY spectrum, and. Thus, the HSQC spectrum revealed the correlation of the hydrogens at δ_H 4.22 and δ_H 5.55 with two oxygenated carbons δ_C 73.3 (CH-2″) and δ_C 62.7 (CH-1″), respectively, together with a *gem*-dimethyl group (δ_H 1.89/δ_C 22.3 and δ_H 1.74/δ_C 27.7), that suggested the presence of an unusual epoxy ring system forming part of a modified prenyl side chain (as found in many instances in *Tephosia* genus) (Table 1). The location of the 1″,2″-epoxy-3″-hydroxy-3″-methylbutyl moiety at C-8 was suggested by the analysis of the HMBC spectrum, which exhibited long-range correlations of the epoxy hydrogens at δ_H 5.55 (H-1″), which revealed transversal peaks with carbon signals at δ_C 73.3 (CH-2″, $^{2}J_{CH}$), 80.5 (C-3″, $^{3}J_{CH}$), 106.1 (C-8, $^{2}J_{CH}$) and 159.8 (C-8a, $^{3}J_{CH}$), and δ_H 4.22 (H-2″) with the aromatic carbon at δ_C 159.8 (C-8a, $^{3}J_{CH}$) and both methyl groups at δ_C 22.3 (CH_3-4″, $^{3}J_{CH}$) and δ_C 27.7 (CH-5″, $^{3}J_{CH}$). Additionally, the correlation between the methoxyl hydrogens observed at δ_H 3.85 with the carbon at δ_C 161.4 confirmed this substituent at C-7 (Fig. 1). The complete analysis of the HMBC spectrum in combination with additional 1D and 2D NMR spectral data and the total ^{1}H and ^{13}C chemical shift assignments was summarized in Table 1 and Fig. 1. Thus, the new flavone was established as 5-hydroxy-8-(1″,2″-epoxy-3″-hydroxy-3″-methylbutyl)-7-methoxyflavone (**1**). The structures of the known compounds, were also determinated by analysis of their spectral data and comparison those reported in the literature.

Compounds **1–4** were tested for cytotoxicity against AGP-01 (cancerous ascitic fluid), HCT-116 (colon adenocarcinoma), HL-60 (leukemia), PC-3 (prostate carcinoma), SF-295 (glioblastoma) and SKMEL 28 (melanoma) cell lines using the MTT assay. Table 2 depicts the absence of cytotoxic activity for all tested compounds against SF-295, HCT-116 and PC-3 cell lines. Compound **1** did not show any activity on cell lines tested. Compound **2** showed high cytotoxic against SK-MEL 28, AGP-01 and HL-60 cells, with IC_{50} values of 5.22, 8.71, and 1.44 μg/ml, respectively. Also, when

Table 2
Cytotoxicity activity against tumor cell lines of compounds (**1**–**4**) isolated from *Tephrosia egregia*.

Compounds	Cell lines					
	SF-295	HCT-116	PC-3	SK-MEL 28	AGP-01	HL-60
1	>20	>20	>20	>20	>20	>20
2	>20	>20	>20	5.22 (4.08–6.68)	8.71 (5.85–12.96)	1.44 (1.11–1.85)
3	>20	>20	>20	15.65 (12.90–18.97)	12.27 (9.83–15.29)	8.10 (6.55–10.02)
4	>20	>20	>20	>20	>20	1.99 (1.52–2.59)

Data are presented as IC_{50} values in μg/ml along with its respective confidence interval of 95% obtained by nonlinear regression from two independent experiments, performed in duplicate, after 72 h of incubation.

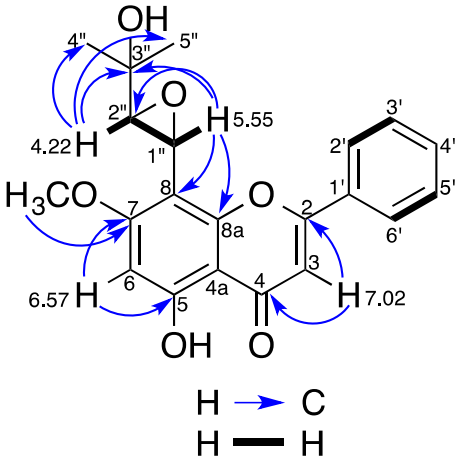

Fig. 1. Selected HMBC and COSY correlations observed for compound **1**.

compared to the compound **2**, compound **3** showed moderate cytotoxic against SK-MEL 28, AGP-01 and HL-60 cells, with IC_{50} values of 15.65, 12.27, and 8.1 μg/ml, respectively. Interestingly, compound **4** showed high cytotoxic only against HL-60 cells, with IC_{50} value of 1.99 (Table 2). Similarly to our results, some authors have shown high cytotoxicity activity of flavonoids from *Tephrosia* genus against colon cancer cell line HT-29 (Ganapaty et al., 2009), but not to melanoma, gastric and leukemia cells.

Compound identification

5-Hydroxy-8-(1″,2″-epoxy-3″-hydroxy-3″-methylbutyl)-7-methoxyflavone (**1**): White solid; mp: 260 °C (decomp); $[\alpha]^{25}_{D}$ = +35.90° (*c* = 0.1; CH_2Cl_2/MeOH 1:1); IR: 3391 cm-1 (OH), 1643 cm^{-1} (C=O), 1207 cm^{-1} (C—O—C). HRESIMS *m/z* = 369.1336 $[M+H]^+$ (calcd. 369.1338). ^1H and ^{13}C spetral data, see Table 1.

Pongaflavone (**2**): White solid; mp 198 °C; $[\alpha]^{25}_{D}$ = +48.23° (*c* = 0.1; CH_2Cl_2); IR: 2974 cm^{-1} (C—H), 1640 cm^{-1} (C=O), 1597 cm^{-1} (C=C, aromatic ring), 1344 cm^{-1} (CH_3), 696 cm^{-1} (monosusbtituted aromatic ring). ^1H NMR (300 MHz, $CDCl_3$) – *δ* = 7.87 (s; H-4′); 7.62 (m; H2′/6′); 7.52 (m; H3′/5′); 6.86 (d; 10; H-4″); 6.72 (s; H-3); 6.34 (s; H-6); 5.63 (d; 10; H-5″); 3.96 (s; H-O-Me-5); 1.51 (s, 4″/6″). EI-MS *m/z* = 334 Da. ^{13}C NMR (75 MHz, $CDCl_3$) – *δ* 160.9 (C-2); 108.9 (C-3); 177.9 (C-4); 160.2 (C-5); 96.9 (C-6); 158.3 (C-7); 102.9 (C-8); 154.2 (C-9); 108.9 (C-10); 131.9 (C-1′); 126.2 (C-2′/6′); 129.2 (C-3′/5′); 131.4 (C-4′); 115.4 (C-4″); 127.8 (C-5″); 78.4 (C-6″); 28.4 (C-4″/6″); 56.7 (C-MeO-5).

Praecansona B (**3**): Yellow oil; IR: 3354.1 cm^{-1} (O—H); 1603 cm^{-1} (C=O); 1116–1099 cm^{-1} (C—O); ^1H NMR (300 MHz, $CDCl_3$) – *δ* = 7.92 (dl, 8.4, H-2/6); 7.42–7.52 (m, H-3/5); 7.42–7.52 (m, H-4); 6.49 (s, H-8); 6.25 (s, H-5′); 5.55 (d; 10; H-3″); 6.54 (d; 10; H-4″); 1.46 (s, H-4″/5″); 3.79 (s, H-OMe-2′); 3.80 (s, H-OMe-6′). ^{13}C NMR (75 MHz, $CDCl_3$) – *δ* 135.3 (C-1); 127.2 (C-2/6); 128.7 (C-3/5); 132.2 (C-4); 182.1 (C-7); 100.7 (C-8); 188.2 (C-9); 114.5 (C-1′); 155.4 (C-2′); 108.2 (C-3′); 156.6 (C-4′); 96.4 (C-5′); 158.6 (C-6′); 77.4 (C-2″); 127.9 (C-3″); 116.7 (C-4″); 28.1 (C-4″/5″); 63.3 (C-OMe-2′); 56.2 (C-OMe-6′).

12a-Hydroxyrotenone (**4**): Yellow oil; IR: 3355 cm^{-1} (O—H); 1610.3 cm^{-1} (C=O); 1457 e 1509 cm^{-1} (C=C); 1088 – 1259 cm^{-1} (C—O); ^1H NMR (300 MHz, $CDCl_3$) – *δ* = 6.57 (s, H-1); 6.48 (H-4); 4.48 (dd, 13, H-6); 4.62 (dd, 13; H-6); 6.53 (d, 8.5, H-10); 7.82 (d, 8.5, H-11); 2.93 (dd, 15.8, H-4′); 3.29 (dd, 15.8, H-4′); 5.23 (t, 9.0, H-5′); 4.92 (s, H-7′); 5.05 (s, H-7′); 1.76 (s, H-8′); 3.72 (s, H-OMe-2); 3.81 (s, H-OMe-3). ^{13}C NMR (75 MHz, $CDCl_3$) – *δ* 109.7 (C-1); 108.8 (C-1a); 144.2 (C-2); 151.3 (C-3); 101.2 (C-4); 148.6 (C-4a); 63.0 (C-6); 157.8 (C-7a); 113.3 (C-8); 168.2 (C-9); 105.5 (C-10); 130.2 (C-11); 112.8 (C-11a); 191.3 (C-12); 67.7 (C-12a); 31.3 (C-4′); 88.1 (C-5′); 143.0 (C-6′); 112.8 (C-7′); 17.24 (C-8′); 56.1 (C-OMe-2); 55.0 (C-OMe-3). EI-MS *m/z* = 410 Da.

Praecansone A (**5**): Yellow oil; ^1H NMR (500 MHz, $CDCl_3$) – *δ* = 7.8 (d, 7.3, H-2/6); 7.3 (d, 7.3, H-3/5); 7.4 (t, H-4); 6.4 (s, H-8); 6.23 (s, H-3′); 5.4 (d, 9.9, H-3″); 6.5 (d, 9.9, H-4″); 1.4 (s, H-(CH_3)-2″); 3.8 (s, H-OMe-2′); 3.8 (s, H-OMe-6′); 3.9 (s, H-OMe-9). ^{13}C NMR (125 MHz, $CDCl_3$) – *δ* 140.2 (C-1); 128.1 (C-2/6); 127.9 (C-3/5); 131.8 (C-4); 190.4 (C-7); 101.1 (C-8); 166.2 (C-9); 111.9 (C-1′); 154.9 (C-2′); 96.3 (C-3′); 155.2 (C-9); 105.2 (C-5′); 158.0 (C-6′); 76.8 (C-2″); 127.0 (C-3″); 117.1 (C-4″); 28.2 (C-5″/6″); 62.4 (C-OMe-2′); 55.8 (C-OMe-6′); 56.3 (C-OMe-9).

2′,6′-Dimethoxy-4′,5′-(2″,2″-dimethyl)-pyranochalcone (**6**): Yellow oil; IR: 2968.3 cm^{-1} (C-H); 1603.3 cm^{-1} (C=O); 1138.4 e 1100.4 cm^{-1} (C—O). ^1H NMR (300 MHz, $CDCl_3$) – *δ* = 7.53–7.56 (m, H-2/6); 7.36–7.44 (m, H-3/5); 7.36–7.44 (m, H-4); 7.44 (d, 16.0, H-7); 7.02 (d, 16.0, H-8); 6.80 (s, H-5′); 5.55 (d, 9.9, H-3″); 6.53 (d, 9.9, H-4″); 1.49 (s, H-4″/5″); 3.74 (s, H-OMe-2′); 3.75 (s, H-OMe-6′). ^{13}C NMR (75 MHz, $CDCl_3$) – *δ* 135.1 (C-1); 128.6 (C-2/6); 129.0 (C-3/5); 130.5 (C-4); 144.9 (C-7); 128.9 (C-8); 194.3 (C-9); 116.5 (C-1′); 155.0 (C-2′); 108.2 (C-3′); 156.2 (C-4′); 95.5 (C-5′); 158.4 (C-6′); 77.1 (C-2″); 127.9 (C-3″); 116.8 (C-4″); 28.1 (C-4″/5″); 63.5 (C-OMe-2′); 56.1 (C-OMe-6′). EI-MS *m/z* = 350 Da.

Pongachalcone (**7**): Yellow crystal solid; mp: 105.2–108.1 °C; IR: 3456.7 cm^{-1} (O—H); 1613.5 cm^{-1} (C=O); 1122.2 e 1146.1 cm^{-1} (C—O). ^1H NMR (300 MHz, $CDCl_3$) – *δ* = 7.60 (m, H-2/6); 7.43 (m, H-3/5); 5.90 (s, H-3′); 5.49 (d, 10.0, H-3″); 7.41 (m, H-4); 6.70 (d, 10.0, H-4″); 7.90 (d, 15.6, H-8); 7.80 (d, 15.6, H-9); 3.90 (s, H-OMe); 1.46 (s, H-5″/6″); 14.50 (s, H-OH). ^{13}C NMR (75 MHz, $CDCl_3$) – *δ* 135.9 (C-1); 106.3 (C-1′); 128.6 (C-2/6); 162.8 (C-2′); 78.5 (C-2″); 129.0 (C-3/5); 91.8 (C-3′); 125.6 (C-3″); 130.2 (C-4); 160.6 (C-4′); 116.3 (C-4″); 103.3 (C-5′); 162.9 (C-6′); 127.9 (C-8); 142.4 (C-9); 192.9 (C-7); 56.1 (C-OMe); 28.6 (C-4″/5″). EI-MS *m/z* = 336 Da.

Maackiain (**8**): Brown amorphous solid; mp: 96.4–98.1 °C; $[\alpha]_D = -196°$ (c. 0.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) – δ = 7.36 (d, 8.4, H-1); 6.56 (dd, H-2); 6.43 (d, H-4); 3.65 (t, H-6); 4.23 (dd, H-6); 3.48 (m, H-6a); 6.72 (s, H-7); 6.44 (s, H-10); 5.47 (d, 6.8, H-11a); 5.90 (s, H-11a); 5.93 (s, H-O-CH_2-O). ^{13}C NMR (125 MHz, $CDCl_3$) – δ 112.8 (C-1a); 132.2 (C-1); 110.0 (C-2); 157.3 (C-3); 103.9 (C-4); 156.9 (C-4a); 66.7 (C-6); 40.4 (C-6a); 118.2 (C-7a); 104.9 (C-7); 141.9 (C-8); 148.3 (C-9); 94.0 (C-10); 154.5 (C-10a); 78.7 (C-11a); 101.5 (C-O-CH_2-O).

p-Cumaric acid (**11**): White solid; mp: 214.2–216.1 °C; ^1H NMR (500 MHz, CD_3OD) – δ = 6.30 (d, 15.7, H-2); 7.59 (d, 15.7, H-3); 7.45 (d, 8.6; H-2′/6′); 6.81 (d, 8.6, H-3′/5′); ^{13}C NMR (125 MHz, CD_3OD) – δ 171.4 (C-1); 115.9 (C-2); 146.4 (C-3); 127.5 (C-1′); 131.1 (C-2′/6′); 116.9 (C-3′/5′); 161.0 (C-4′).

Cinnamic acid (**12**): White solid; mp: 168.1–170.2 °C; IR: 3436.90 cm^{-1} (O—H); 2933.91 cm^{-1} (O—H of carboxilic acid); 1692.3 cm^{-1} (C═O); 1625.83 (C—O); ^1H NMR (500 MHz, CD_3OD) – δ = 6.30 (d; 15.8; H-2); 7.59 (d; 15.8; H-3); 7.16 (d; 1.6; H-2′); 6.81 (d; 8.2; H-5′); 7.04 (dd; 8.2; 1.6; H-6′); 3.88 (s, H-OMe-3′). ^{13}C NMR (125 MHz, CD_3OD) – δ 171.1 (C-1); 116.0 (C-2); 147.0 (C-3); 127.9 (C-1′); 111.8 (C-2′); 149.5 (C-3′); 150.6 (C-4′); 116.6 (C-5′); 124.1 (C-6′); 56.6 (C-O-Me-3′).

Conclusions

In the present phytochemical investigation, the constituents of *T. egregia* have been examinated, and here the isolation a new flavonoid, 5-hydroxy-8-(1″,2″-epoxy-3″-hydroxy-3″-methylbutyl)-7-methoxyflavone (**1**) is reported. In addition, ten known compounds were also isolated. Antiproliferative effects (IC_{50}, μg/ml) is present only in the (2″,2″-dimethyl)-pyranochalcones (**2–4**), that exhibited mild to moderate activity. On the contrary, compound **1** was inactive on these tumor cells lines. Interestingly, compound **4** showed a selective effect only against HL-60 cells, with an IC_{50} of 1.99 μg/ml. In general, HL-60 was the most sensitive cell line, to which three of the four tested compounds presented IC_{50} values below 20 μg/ml.

In summary, the present study is in agreement that *Tephrosia* genus is a rich source of phenolic compounds, so this results can be used in further pharmacological investigations.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

MVST and JQL (MSc and PhD students, respectively), IGP, ACM, DCCF (undergraduate students) contributed running the laboratory work and drafted the paper; AMCA, MCFO, RBF, GMPS did the analysis and interpretation of data of RMN, critical revision of the manuscript; ATAP, MASL and FRLS carried out the separation on HPLC of pure compounds. PRVR acquisition and interpretation of Mass spectra. RCM, DDR, MMB and MEAM conducted and interpreted the cytotoxic assay. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bjp.2018.03.008.

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