

Antitumor screening of crude extracts of ten medicinal plants of *Polygala* genus from Southern Brazil

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ABSTRACT

In this study crude extracts from 10 species among of the 39 *Polygala* L. genus found in the South region of Brazil, were subjected to screening for antitumor activity against the tumor cell lines, HL-60 (myeloid leukemia), Jurkat (lymphoblastic leukemia), THP-1 (monocytic leukemia), MCF-7 and MDA-MB-231 (breast carcinoma), HCT-116 (colorectal carcinoma) and Vero (kidney of green monkey, used as a non-tumor cell model). The evaluation was carried out using the MTT assay. Only the extracts of *Polygala densiracemosa*, *Polygala campestris* and *Polygala molluginifolia* inhibited the tumor cell viability by more than 50%. The best results were observed for *P. molluginifolia*, with inhibition of the cell viability of HL-60 (83%), Jurkat (78%), MDA-MB-231 (75%), MCF-7 (70%) and HCT-116 (64%), however this species also inhibited the viability of the Vero cells (32%). The *P. campestris* species showed selectivity for Jurkat (66%) and HL-60 (78%) cells whilst *P. densiracemosa* showed selectivity only for HL-60 cells (66%). In addition, the toxicity toward non-tumor cells was low (11% for *P. campestris* and 12% *P. densiracemosa*), showing that these species are promising sources for obtaining candidates for chemotherapy drugs.

INTRODUCTION

The *Polygala* L. genus is the most abundant of the *Polygalaceae* family, with around 725 species distributed worldwide in many different environments. In southern Brazil 39 species of this genus have been identified (Ludtke *et al.*, 2013). The *Polygala* genus is characterized by the accumulation of coumarins, flavonoids, polysaccharides, sterols, styrylpyrones, triterpenic saponins and xanthenes (Klein Júnior *et al.*, 2012). Several *in vitro* and *in vivo* studies have highlighted the important biological effects of species of the genus *Polygala*, for instance: the anti-inflammatory activity of sterols from *Polygala sabulosa* (Borges *et al.*, 2014), isoflavones from *Polygala*

molluginifolia (Arruda-Silva *et al.*, 2014) and triterpenic saponins from *Polygala tenuifolia* (Cheong *et al.*, 2011); the antitumor activity of polysaccharides from *Polygala tenuifolia* (Xin *et al.*, 2012; Zhang *et al.*, 2015) and xanthenes from *Polygala hongkongensis* (Wu *et al.*, 2011); the antidepressant activity of styrylpyrones from *Polygala sabulosa* (Capra *et al.*, 2010); and the antinociceptive activity of flavonoids from *Polygala paniculata* (Lapa *et al.*, 2009). Currently, chemotherapy is the main procedure for the treatment of cancer and it induces serious side effects on patients. Extensive studies seeking new active plant extracts that can be used in the treatment of cancer have been carried out in the search for drug candidates that have high efficacy and safety (Gao *et al.*, 2005; Ni *et al.*, 2010). This paper describes the screening of extracts of *Polygala* L. spp. for antitumor activity against cell lineages of different types of leukemias and carcinomas using the MTT cell viability assay.

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MATERIALS AND METHODS

Plant material

The species *Polygala campestris* (January 2012), *Polygala linoides* (September 2014) and *P. sabulosa* (September 2014) (deposited at the Herbarium FLOR – UFSC, under numbers 39195, 55317 and 55316, respectively) were collected in Rancho Queimado, Santa Catarina State. *Polygala altomontana* was collected in Bom Jardim da Serra, Santa Catarina State (October 2014) and deposited at the Herbarium FLOR – UFSC, under number 55186. *Polygala longicaulis* was collected in Ponta Grossa, Paraná State (December 2010) and deposited at the Herbarium FLOR – UFSC, under number 38271. *Polygala molluginifolia* was collected in Ponta Grossa, Paraná State (January 2012) and deposited at the Herbarium FLOR – UFSC, under number 48690. *Polygala densiracemosa*, *Polygala lancifolia* and *Polygala pulchella* (deposited at the Herbarium CTBS – UFSC, under numbers 5009, 5005 and 5006, respectively) were collected in September 2014 in Urubici, Santa Catarina State. *Polygala paniculata* (deposited at the Herbarium UPCB – UFPR, under number 26027) was collected in February 2013 in Florianópolis. The plants were identified by Dr. Rafael Trevisan, Department of Botany at UFSC, Dr. Olavo de Araújo Guimarães, Department of Botany at UFPR and Dr. José Floriano Barêa Pastore, Department of Botany at UFSC - Curitiba.

Crude extracts

The dried and powdered whole plants were extracted by exhaustive maceration (three times for seven days each time) with EtOH 96° GL at room temperature. The resulting extracts were combined, filtered and concentrated under reduced pressure at 50 °C to yield the crude hydroalcoholic extract. The percentages of crude extract (relative to the dried plant material) obtained were: *P. campestris* (18.8%), *P. linoides* (11.5%), *P. sabulosa* (23.6%), *P. altomontana* (18.0%), *P. longicaulis* (29.0%), *P. molluginifolia* (27.2%), *P. densiracemosa* (17.1%), *P. lancifolia* (13.0%), *P. pulchella* (13.0%) and *P. paniculata* (6.9%).

Cell lines

HL60 (wild-type human promyelocytic leukemia), Jurkat (human immortalized line of T lymphocyte) and THP-1 (human monocytic cell line derived from an acute monocytic leukemia patient) cell lines were donated by Dr. Gustavo Amarante-Mendes (São Paulo University, Brazil). MCF-7 and MDA-MB-231 (human breast carcinoma) cell lines were donated by Dr. Alfredo Goes (Federal University of Minas Gerais, Brazil), and HCT-116 (human colorectal carcinoma) cells were donated by Dr. Ricardo Luis A. Silva, Cancer Institute – INCA/Brazil. Vero cells (derived from the kidney of an African green monkey) were donated by Erna Kroon (Federal University of Minas Gerais, Brazil).

All lineages were maintained in the logarithmic phase of growth in RPMI 1640 or DMEM (Dulbecco's Modified Eagle Medium) supplemented with 100 U mL⁻¹ penicillin and 100 mg mL⁻¹ streptomycin (GIBCO BRL, Grand Island, NY) enriched

with 2 mM of L-glutamine and 10% (leukemic cells) or 5% (adherent cells) of fetal bovine serum. All cultures were maintained at 37 °C in a humidified incubator with 5% CO₂ and 95% air. The media were changed twice weekly and they were regularly examined.

Evaluation of the cytotoxic effect against human tumor cell lines

Cells were seeded at densities/well of 50,000 for HL-60 cells and 100,000 for Jurkat and THP-1 cells. Adherent cells were inoculated at 10,000 cells/well. The plates were pre-incubated for 24 h at 37 °C to allow adaptation of the cells prior to the addition of the extracts. Freshly prepared solutions of the different extracts were screened at 50 µg mL⁻¹ in DMSO. Subsequently, the plates were inoculated for 48 h in an atmosphere of 5% CO₂ and 100% relative humidity. Control groups included treatment with 0.5% DMSO (negative control) and etoposide (10 µM, positive control). Cell viability was estimated by measuring the rate of mitochondrial reduction of MTT. All substances were dissolved in dimethylsulfoxide (DMSO) prior to dilution, with a final cell concentration of 0.5%. All extracts were tested in triplicate, in two independent experiments.

In vitro cell viability assay - MTT assay

Cell viability was evaluated using the MTT assay as described by Mosmann (1983). Briefly, after 4 h of incubation of the cells with the different extracts, 20 µL of MTT solution (5 mg mL⁻¹ in phosphate-buffered saline) were added to each well, the supernatant was removed and 200 µL of 0.04 M HCl in isopropyl alcohol were added to dissolve the formazan crystals. The optical densities (OD) were evaluated using a spectrophotometer at 595 nm. Controls included drug-containing medium (background) and extract-free complete medium. Extract-free complete medium was used as the control (blank) and was treated in the same way as the extract-containing media. Results were expressed as percentage of cell proliferation, compared with the 0.5% DMSO control and were calculated as follows: viability (%) = (mean OD treated culture – mean OD background)/(mean OD untreated culture, i.e. 0.5% DMSO – mean OD blank wells) x 100. Interaction of the extract with the medium was estimated on the basis of the variations between the drug-containing medium and the extract-free medium to avoid false-positive or false-negative results in relation to the control (Ulukaya *et al.*, 2004).

RESULTS AND DISCUSSION

In the experiments six tumor cell lines and one line of non-tumor cells (Vero) were evaluated. This latter lineage has been used in toxicity studies as a predictive model of normal cells (Freire *et al.*, 2008). Three cell lines were derived from colorectal carcinoma (HCT-116) and two from breast cancer cells: an estrogen-dependent lineage represented by MCF-7 (responsive to chemotherapy) and an aggressive and unresponsive estrogen-independent lineage MDA-MB-231 (Srisawat *et al.*, 2014).

Table 1: Effect of crude plant extracts on proliferation/ viability of tumor cells and non-tumor cells (Vero).

Extracts	% inhibition of proliferation						
	HL-60	MCF-7	MDA-MB-231	JURKAT	HCT-116	THP-1	VERO
<i>P. altomontana</i>	10.37 ± 3.70	8.55 ± 2.72	19.55 ± 4.80	34.21 ± 4.28	#	#	#
<i>P. campestris</i>	78.57 ± 0.47	30.47 ± 2.32	#	66.05 ± 4.43	#	49.26 ± 4.82	11.69 ± 4.90
<i>P. densiracemosa</i>	66.24 ± 1.01	11.87 ± 3.34	20.37 ± 4.49	52.36 ± 0.34	#	28.36 ± 4.05	12.64 ± 3.57
<i>P. lancifolia</i>	#	12.65 ± 4.73	6.57 ± 1.33	2.48 ± 2.59	#	#	#
<i>P. linooides</i>	7.13 ± 3.17	8.69 ± 2.37	7.97 ± 4.57	28.43 ± 4.48	#	#	#
<i>P. longicaulis</i>	#	13.92 ± 3.16	11.75 ± 3.11	#	#	9.21 ± 4.28	13.14 ± 0.17
<i>P. molluginifolia</i>	83.26 ± 0.67	69.98 ± 0.17	75.04 ± 0.37	78.39 ± 0.22	64.26 ± 4.89	35.03 ± 4.35	32.54 ± 4.65
<i>P. paniculata</i>	46.05 ± 4.38	13.85 ± 3.56	23.14 ± 4.36	55.96 ± 4.29	#	7.94 ± 0.07	#
<i>P. pulchella</i>	30.56 ± 1.95	3.96 ± 0.07	16.48 ± 4.07	31.23 ± 1.83	#	#	#
<i>P. sabulosa</i>	#	#	6.71 ± 0.93	12.27 ± 1.60	#	#	#
Etoposide*	71.06 ± 4.81	27.93 ± 4.23	6.25 ± 4.85	39.33 ± 5.01	29.65 ± 4.13	72.37 ± 4.61	21.28 ± 1.31

*positive control 10 µM; # stimulated cell proliferation.

To represent models of relevant leukemias with an impact on public health, cells derived from the human promyelocytic leukemic (HL-60 cells), acute T-lymphocytic leukemia (Jurkat) and acute monocytic leukemia (THP-1) were used. The cell lines were evaluated by the MTT method in which the tetrazolium salt is reduced to purple formazan crystals mainly by mitochondrial succinate dehydrogenase and the color intensity of the formazan dye is correlated to the number of viable cells (Mosmann, 1983). The results demonstrate that only *P. densiracemosa*, *P. campestris* and *P. molluginifolia* inhibited by more than 50% the proliferation of tumor cells and presented low inhibition of the proliferation of non-tumor cells (Figure 1 and 2), which is a parameter for antitumor screening (Senthilraja *et al.*, 2015). The *P. densiracemosa* sample was active only in the inhibition of the HL-60 strain (66%) and Vero cell viability (12%), suggesting a possible selectivity for myeloid leukemia cells and low toxicity against non-tumor cells. The *P. campestris* sample inhibited the viability of Jurkat (66%) and HL-60 (78%) cells, with low toxicity toward Vero cells (11%). The *P. molluginifolia* sample inhibited by over 50% the proliferation in 5 of the 6 tumor cell lines tested: HL-60 (83%), Jurkat (78%), MDA-MB-231 (75%), MCF-7 (70%) and HCT-116 (64%). However, the Vero cell viability was inhibited by 32%, demonstrating cytotoxicity also toward non-tumor cells (Figure 1 and Table 1). Isoflavones are the main secondary metabolites of *P. molluginifolia* (Venzke *et al.*, 2013), which may be related to the pronounced anti-tumor activity of this species (De la Parra *et al.*, 2016).

Polygala campestris and *P. densiracemosa*, in preliminary studies, showed the accumulation of xanthenes (unpublished data), which may explain the cell inhibition profile (HL-60 and Jurkat) and low toxicity against non-tumor cells. Previous studies have demonstrated the potent activity of xanthenes isolated from *P. hongkongensis* against three tumor cell lines (HepG2 human hepatocellular carcinoma cells; GLC-82 human lung carcinoma cells and MCF-7 human breast carcinoma cells), using the MTT assay (Wu *et al.*, 2011). In another study, the antitumor activity of a root extract of *Polygala senega* was demonstrated (Paul *et al.*, 2010).

One of the important criteria for a candidate antitumor drug is to have minimal or no side effects on the normal cells of patients undergoing chemotherapy. One way to achieve this is by employing lower doses of the drug. This invariably means that the drug should not only have potent activity, but should also display a high degree of selectivity (Badisa *et al.*, 2009).

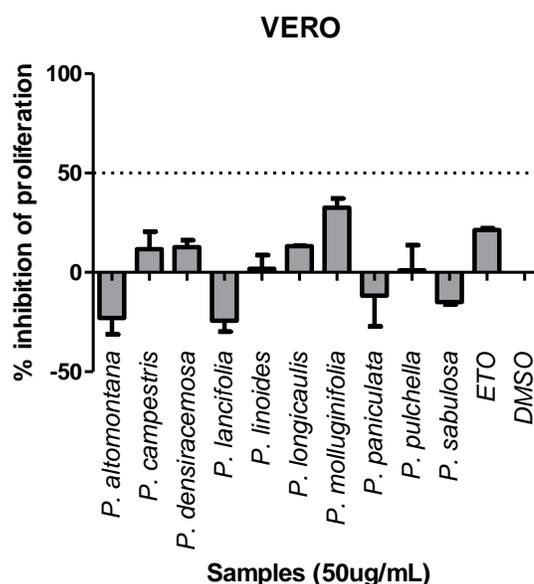


Fig. 1: Effect of crude plant extracts on proliferation/ viability of non-tumor cells (Vero). Data representative of two independent experiments performed in triplicate. Control groups included treatment with 0.5% DMSO (negative control) and etoposide (10 µM, positive control).

The *P. campestris* sample showed selectivity for cell lines of lymphoid and myeloid leukemia while *P. densiracemosa* showed selectivity only for myeloid cells, and both species presented low toxicity toward non-tumor cells. The species *P. molluginifolia* demonstrated the highest inhibition values for tumor cells, as can be seen in Figure 2, where 83% cell proliferation can be observed for HL-60 cells, suggesting a potent antitumor activity. However, this species also inhibited the viability of non-tumor cells lines by 32%.

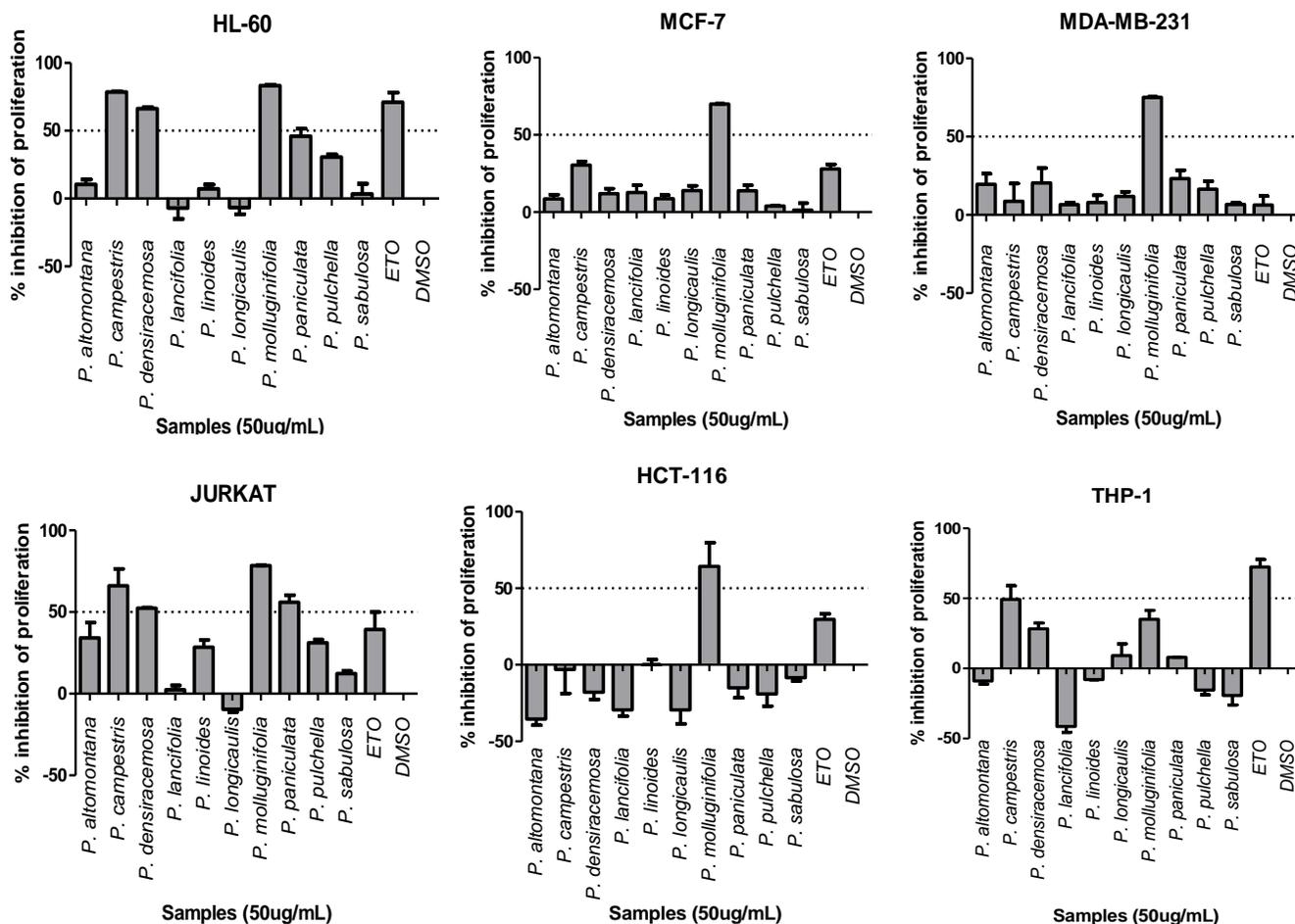


Fig. 2: Effect of crude plant extracts on proliferation/ viability of tumor cells. Data representative of two independent experiments performed in triplicate. Control groups included treatment with 0.5% DMSO (negative control) and etoposide (10 μ M, positive control).

CONCLUSIONS

The best results found for antitumor screening were observed for the *P. molluginifolia* sample, but this species also inhibited the viability of Vero cells.

The species *P. campestris* showed selectivity for Jurkat and HL-60 cells whilst *P. densiracemosa* showed selectivity only for HL-60 cells, with low toxicity toward non-tumor cells. These results demonstrate that *P. densiracemosa* and *P. campestris* are promising sources for obtaining candidates for chemotherapy drugs.

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