

Evaluation of the Antiproliferative Effect of Infusions and Essential Oil of *Aloysia gratissima*

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Abstract: *Aloysia gratissima* is used in Brazilian folk medicine for the treatment of digestive and respiratory diseases. The infusions (6 and 24 g L⁻¹) and essential oils (0.25%, on ethanol) were prepared and we used groups of five *Allium cepa* bulbs for each treatment. A total of 2500 cells per treatment were analyzed and the mitotic indexes were calculated. The antiproliferative effect of infusions and essential oils of *Aloysia gratissima* on the *Allium cepa* (onion) cell cycle was evaluated using the leaves of studied specimens. The infusions presented a significant decrease in the mitotic index (4.55% at 6 g L⁻¹ and 2.04% at 24 g L⁻¹) compared to the control-water (6.83%), as well as for the essential oil (2.58%), in comparison to the control-ethanol (3.65%). This investigation showed that the infusions and essential oil of *Aloysia gratissima* present important antiproliferative effects on the *Allium cepa* cell cycle.

Key words: *Allium cepa*, *Aloysia gratissima*, aqueous extracts, essential oil, antiproliferative effect

INTRODUCTION

The knowledge about medicinal plants represents, in some cases, the only therapeutic treatment for many communities. However, the indiscriminate use of some species can be prejudicial to the health of the population and the effect of the action of extracts of plants on cell division in other organisms is important. The possible adverse and mutagenic effects can be detected by inhibiting cell division, like chromosomal breakages in metaphases, anaphasic bridges and micronuclei in telophase (Vieira and Vicentini, 1997).

The World Health Organization estimates that up to 80% of the world's population relies on traditional medicinal system for some aspect of primary health care (Farnsworth *et al.*, 1985). In developing or non-industrialized countries, like Brazil, the use of infusions of plants for treatment of diseases is common (Teixeira *et al.*, 2003).

Essential oils are obtained from many aromatic plant species and are used in industries for the production of soaps, cosmetics, perfumes and foods. Investigations concerning the evaluation of the biological activities of

essential oils of some medicinal plants have revealed that some exhibited antibacterial, antifungal, anti-inflammatory, analgesic and insecticidal properties (Burt, 2004; Bakkali *et al.*, 2008). They are usually used topically, in massages, therapeutic baths, compresses, or inhalation (Steflitsch and Steflitsch, 2008).

Aloysia gratissima Gilles and Hook Tronc. (Verbenaceae), commonly known as erva-santa, alfazema-do-brasil, erva-da-graça and erva-de-nossa-senhora, is an herb used in Brazilian popular medicine, especially in the Southern state of Rio Grande do Sul. Traditionally, it is used as gastrointestinal and respiratory medicine (Pascual *et al.*, 2001). The tea made from its leaves is popularly used as an analgesic, antifu, anti-rheumatic, balsamic and stomaquic, to decrease the arterial pressure and cholesterol values, as well as for pneumatic diseases.

Investigations with this plant demonstrated high content of essential oil and differences in principal chemical composition (Agostini *et al.*, 2003; Ricciardi *et al.*, 2005; Franco *et al.*, 2007).

The use of medicinal plants for the treatment of diseases is an exploratory practice and widespread in

Brazil. Considering the extensive medicinal use of *Aloysia gratissima*, studies using bioindicators of toxicity and of mutagenicity, with *Allium cepa* test *in vivo* are necessary.

Based on this context, the purpose of this study was to evaluate the antiproliferative effect of infusions and essential oil of *A. gratissima* using the *A. cepa* cell cycle.

MATERIALS AND METHODS

Plant material: The leaves of *A. gratissima* were collected in Santa Maria (Rio Grande do Sul), Brazil, in July 2007. A voucher specimen was deposited in the Herbarium SMDB (Santa Maria Department of Biology), at UFSM, in Santa Maria, Rio Grande do Sul, Brazil.

Preparation of the aqueous extracts and essential oil: Fresh leaves of *A. gratissima* were infused in water for 5 min and the aqueous extracts were strained and placed at room temperature for cooling. These infusions were prepared in two concentrations: 6 and 24 g L⁻¹. The 6 g L⁻¹ is a normal concentration used during the preparation of the medicinal tea. The essential oil was obtained from fresh leaves of *A. gratissima* by hydro-distillation with Clevenger's apparatus. The oil concentration used in the test was 0.25% (w/v) in ethanol.

Evaluation of antiproliferative effect on *Allium cepa* assay: For the meristematic onion root-tip cell test, we used 25 *A. cepa* bulbs divided into five groups of five onion bulbs for each treatment (distilled water, ethanol, aqueous extracts at 6 and 24 g L⁻¹ and essential oil at 0.25%). For each treatment, all bulbs were rooted in distilled water for three days and then they were placed in their respective treatment for 24 h. The two control groups, which had not received treatment, remained in distilled water and ethanol, respectively. After 24 h, controls and experimental bulbs were collected and fixed in 3:1 (v/v) ethanol-acetic acid for 24 h. Subsequently, they were placed in 70% (v/v) aqueous ethanol under refrigeration (4±2°C) until analyzed (Fiskesjö, 1994; Knoll *et al.*, 2006). For each bulb, five slides were made using five root-tips hydrolyzed in 1 N HCl for 5 min and washed in distilled water. The fragmented meristematic regions were stained with 2% (w/v) acetic orcein (Guerra and Souza, 2002). Each slide was assessed by bright-field optical microscopy at 500x magnification and the number of interphase, prophase, metaphase, anaphase and telophase cells was recorded. At least 2500 cells for each treatment and the controls were scored. Mean values for the different cell cycle phases and the mitotic index (%) were calculated.

Table 1: No. of cells in different phases of the cell cycle and the mitotic index of onion root-tip cells. The total number of cells analyzed for each treatment was 2500

Treatments	Interphase	Divisions (prophase, metaphase, anaphase and telophase)	Mitotic index (%)*
Control in water	2340	160	6.83 ^a
Aqueous extract at 6 g L ⁻¹	2391	109	4.55 ^b
Control in ethanol	2412	88	3.65 ^c
Essential oil 0.25%	2437	63	2.58 ^d
Aqueous extract at 24 g L ⁻¹	2450	50	2.04 ^e

*Means, in column, with the same letter are not significantly different (χ^2 test, $p \leq 0.05$)

Statistical analysis: The statistical data analysis was performed using the Chi-squared (χ^2) test at $p \leq 0.05$, with the statistical program BioEstat (Version 3.0) (Ayres *et al.*, 2003).

RESULTS

Table 1 shows the total number of analyzed cells and the number of cells observed in interphase and in the different phases of cell division during the *A. cepa* cell cycle. In this same table, the mitotic index (number of cells in division/number of cells in interphase) is also demonstrated.

The mitotic index of the *A. cepa* cell cycle decreased significantly after 24 h in both concentrations of aqueous extracts and in the essential oil of *A. gratissima* when compared to the control group-distilled water. The values obtained were: 4.55% for aqueous extract at 6 g L⁻¹ ($\chi^2 = 10.22$); 2.04% for aqueous extract at 24 g L⁻¹ ($\chi^2 = 60.14$) and 2.58% for essential oil ($\chi^2 = 44.16$). Between the control group-ethanol and treatments, significant differences were also observed (aqueous extract at 6 g L⁻¹, $\chi^2 = 2.33$; aqueous extract at 24 g L⁻¹, $\chi^2 = 10.76$; essential oil, $\chi^2 = 4.27$). Likewise, significant difference was also obtained between the control groups (distilled water and ethanol) ($\chi^2 = 21.99$). After analyzing the three treatments, significant differences in the mitotic index were obtained: aqueous extract at 6 g L⁻¹ x aqueous extract at 24 g L⁻¹ x essential oil ($\chi^2 = 26.76$); aqueous extract at 6 g L⁻¹ x aqueous extract at 24 g L⁻¹, ($\chi^2 = 22.61$); aqueous extract at 6 g L⁻¹ x essential oil ($\chi^2 = 12.74$); aqueous extract at 24 g L⁻¹ x essential oil ($\chi^2 = 1.53$).

DISCUSSION

The cytotoxic and genotoxic effects of aqueous extracts and essential oils of *A. gratissima* on *A. cepa* were evaluated and we observed that the teas and essential oil significantly inhibited cell division, in both analyzed concentrations (Table 1). This result indicates

the existence of antiproliferative activity in the tea made from this species. Comparatively, similar results were obtained from the essential oil and aqueous extracts at 24 g L⁻¹, though the tea presented higher potential of inhibiting cell division. Considering that this difference is significant, but the values are numerically adjacent, we accept the hypothesis that substances present in the leaves act in inhibiting cell division.

According to the results observed in Table 1, it is important to consider essential oil composition and to indicate the existence of a relationship with the increase of the content of leaves used in tea preparation. However, it is difficult to compare the qualitative and quantitative chemical composition present in the samples, due to the extraction processes used in this study (aqueous extract and essential oil). Da Silva *et al.* (2006) evaluated the chemical composition of the aerial parts of *A. gratissima* and the results demonstrated the presence of sesquiterpenoids compounds (guaiol, bisabolol and spathulenol), triterpenoids (α -amirin, betulinic acid, oleanolic acid and ursolic acid), flavonoids and phenylethanoids (verbascoside and arenarioside).

The literature points the chemical composition of the essential oil of *A. gratissima* and that differences were observed, due to the site of collection, environmental conditions and genetic variable. Duschatzky *et al.* (2004) evaluated the essential oil composition of the species collected from Argentina and the main components were: cadinol (17.4%), caryophyllene oxide (15.8%), limonene oxide (5.3%), chrysanthenyl acetate (5.6%) and β -caryophyllene (4.8%); in another research, the chemical composition of aerial parts of *A. gratissima* in three localities of the province of Corrientes (Argentina), during different seasonal stages was studied and the principal constituents were: β -elemene (tr to 35.7%), viridiflorol (0.9-33.6%), β -caryophyllene (1.8-28%), α -thujone (6.8-17.5%), 10-epi-cubebol (0.1-13.4%), bicyclogermacrene (3.8-12.8%), (E)-nerolidol (tr to 11.6%) and germacrene D (1.9-10.1%). The variation was associated with the ontogeny of the plant population and the geographical origin of the material (Ricciardi *et al.*, 2005). The analysis of the constituents present in essential oils of plant collected from Goiânia State (Brazil) were evaluated by Franco *et al.* (2007) and found the principal components to be β -pinene (14.06%), trans-pinocamphone (18.42%), trans-pinocarvila acetate (13.55%), pinocamphone (6.59%), guaicol (6.27%), β -caryophyllene (4.99%) and caryophyllene oxide (4.15%). Through these observations it is difficult to state which compound present in the oil is responsible for the decrease in the mitotic index values obtained from meristematic cells of *A. cepa* analysis, but the presence of

antioxidative substances can be related to that activity. The antiproliferative capacity of other medicinal species that present antioxidative substances in their composition has been studied, as shown for *Pterocaulon polystachyum* DC, *Achyrocline satureoides* (Lam.) DC, *Psychotria myrnanthia* Mull. Arg. and *P. leiocarpa* Cham. et Schlecht (Knoll *et al.*, 2006; Fachinnetto *et al.*, 2007; Lubini *et al.*, 2008).

The cytotoxicity tests, employing a plant test *in vivo*, such as the *A. cepa* test, are validated by several researchers with a good correlation to the *in vitro* test using animal cells (Vicentini *et al.*, 2001; Teixeira *et al.*, 2003).

The mitotic index is used as an indicator of adequate cell proliferation (Gadano *et al.*, 2002), which can be measured using the *A. cepa* plant system. The chromosomal aberration method in *A. cepa* roots is validated by the International Programme on Chemical Safety (IPCS, WHO) and the United Nations Environmental Programme (UNEP), as a test efficient for the analysis and monitoring *in situ* of genotoxicity of environmental substances (Silva *et al.*, 2004).

In this study, our results demonstrated that infusions and the essential oil of leaves of *A. gratissima* showed high antiproliferative activity using the *in vivo A. cepa* cell test, indicating their potential use for inhibiting the cell cycle in tumors and anti-aging. It should be noted that there are no works that demonstrate the antiproliferative activity of the plant. However, this study is bioindicative and more research will be necessary to establish the use of extracts of *A. gratissima* as antimutagenic in humans.

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