Anais da Academia Brasileira de Ciências (2010) 82(4): 857-860
(Annals of the Brazilian Academy of Sciences)
ISSN 0001-3765
www.scielo.br/aabc

Alterations in the mitotic index of *Allium cepa* induced by infusions of *Pluchea sagittalis* submitted to three different cultivation systems

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Manuscript received on August 11, 2009; accepted for publication on January 25, 2010

ABSTRACT

We evaluated the antiproliferative effect of infusions from *Pluchea sagittalis* using the *Allium cepa* test. Infusions in three concentrations (2.5, 5, and 25 g dm^{-3}) of leaves cultivated in three environments (*in vitro*, acclimatized growth chamber, and field) were used. Six onion bulbs were used for each of the eight treatments, and the mitotic index was obtained from 6000 cells per treatment. In conclusion, leaf infusions of *P. sagittalis* cultivated in the field have a high antiproliferative activity, as well as the cultivation system influences the antiproliferative potential.

Key words: antiproliferative effect, camphorweed, cell cycle, medicinal plant, mutagenicity.

Leaf infusions of *Pluchea sagittalis* (Lam.) Cabrera have been traditionally used in South American popular medicine as a chest, carminative, and stomach agent (Lorenzi and Matos 2002). Experimental studies have demonstrated that extracts of this species present a potent antioxidant action and inhibit the expression of the protein Hsp72, which is responsible for inducing oxidative stress (Pérez-García et al. 2001).

Allium cepa has been used to evaluate DNA damages, like chromosomal aberrations and disturbances in the mitotic cycle (Leme and Marin-Morales 2009), and the *Allium cepa* test is considered extremely efficient for analyzing and monitoring *in situ* the genotoxicity of several substances (Silva et al. 2004).

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Phytochemical studies with *P. sagittalis* demonstrate the presence of various secondary metabolites (Guilhon and Müller 1996), where many can present bioactivity in cells of different organisms (Queiroz et al. 2001).

The aim of this study was to evaluate the antiproliferative effect of infusions with plant leaves of *P. sagittalis* cultivated in three contrasting environments by the *Allium cepa* test.

Aseptic plants were used as sources of explants for *in vitro* plants. Nodal segments (1cm) were cultivated in MS medium (Murashige and Skoog 1962), supplemented with 6g dm^{-3} of agar, 30g dm^{-3} of sacarose, and 100mg dm^{-3} of mio-inositol. The cultivation was carried out in a growth chamber for 120 days at $25 \pm 2°C$, photoperiod of 16/8 h light dark cycle with a luminous

intensity of 35μmol m^{-2} s^{-1} by fluorescent light bulbs. Two-month-old *P. sagittalis* plants, obtained from the *in vitro* culture, were submitted to a process of acclimatization. For plants cultivated in the field, approximately two-month-old plants in vegetative stage were collected from areas around the UFSM campus.

The infusion preparation was undertaken with fresh leaves from plants of *P. sagittalis* cultivated in three contrasting environments. These were placed in boiling water for 10 min, strained and placed to cool. The follow treatments were used: 2.5, 5, and 25g dm^{-3} of *P. sagittalis* leaves, three systems of *P. sagittalis* cultivation, and two controls (negative was water and positive was $CuSO_4$ at 0.2g dm^{-3}).

Six onion bulbs were placed in distilled water to root for each treatment; subsequently, they were transferred to the infusions for 24 h and to the positive and negative controls. Afterwards, the rootlets were collected, fixed in ethanol-acetic acid (3:1), and conserved in ethanol 70%.

The slides were prepared by the squash technique (Guerra and Souza 2002) and stained with acetic orcein 2%. The analysis of 1000 cells per bulb in the mitotic phases was carried out, totalizing 6000 cells per treatment. The mean values of cell numbers in each of the phases in the onion cell cycle were observed, determining the rate of the mitotic indices. The data were statistically analyzed by the Tukey test, ($p<0.05$).

In Table I the number of cells in division, as well as the mitotic index obtained from cells observed, are shown.

For extracts of plant leaves cultivated in the growth chamber, the concentration of 25g dm^{-3} reduced the MI in 40%, while the concentrations of 2.5 and 5g dm^{-3} did not demonstrate any difference compared to the negative control. The most interesting effect on the MI was detected by the extract of plants cultivated in the field where, at the concentration of 25g dm^{-3}, a reduction equivalent to that obtained by the positive control was observed. These results demonstrate that only in concentrations of the extract corresponding to 10 times that one used in the popular infusion form occurred a significant reduction in MI.

The onion test system has been used as a bioindicator of genotoxicity for several medicinal tea plants, like *Pterocaulon polystachyum* (Knoll et al. 2006), *Achyrocline satureioides* (Fachinetto et al. 2007), and two *Psychotria* spp. (Lubini et al. 2008).

In this study, the effect of leaf infusions on the antiproliferative capacity was also strongly dependent on the origin of the leaves. The plants cultivated in the field proportioned a larger reduction in the MI when compared to those cultivated in the growth chamber. Nevertheless, *in vitro* cultivation did not present a decrease of effect. The results of the present study suggest that the antiproliferative capacity obtained by the infusion of plant leaves cultivated *in vitro* can be attributed to the smallest concentration or type of bioactive substances.

There are great differences between the environments used to cultivate *in vitro* and *ex vitro*, especially the illumination (quality and quantity), relative air humidity, nutrients, gas composition, and substrate types (Hazarika 2003). Differences between these two cultivation environments and their effects on plant growth and development have previously been found in several studies (Pospísilová et al. 1999, Hazarika 2003). Lavola et al. (1997) observed that *Betula pendula* plants submitted to growing levels of type β ultraviolet light presented significant increases in the concentrations of some flavonoids like quercitrin, mycetin-3-galactoside, and chlorogenic acid. Besides this, the concentration of phenolic compounds in the leaves was altered during the growth season, but this change was not due to an increase in ultraviolet light. In a study carried out with carqueja (*Bacharis trimera*), a greater concentration of flavonoids was observed during the summer harvest (Borella et al. 2001). Many factors, like the soil composition, hydric stress, temperature, humidity, ultraviolet radiation, and attack of pathogens, can affect the synthesis and accumulation of secondary metabolites, like tannins and flavonoids (Gobbo-Neto and Lopes 2007).

Further studies are important to fraction leaf extracts of this species, as well as to test the secondary metabolites as isolates on their proliferative capacity of several live systems, like the onion test system.

TABLE I
Number of cells in interphase and mitosis, as well as the mitotic index of roots of *Allium cepa* treated with infusions of *Pluchea sagittalis*.

Cultivation system	Extract [g dm^{-3}]	Cell in interphase	Cells in division	Mitotic index (%)
Negative control (H_2O)		5651	349	6.19 a[1]
Positive control ($CuSO_4$)		5934	66	1.11 c
in vitro	2.5	5690	310	5.45 ab
	5	5676	324	5.71 ab
	25	5720	280	4.83 ab
Acclimatized growth chamber	2.5	5656	344	6.09 a
	5	5714	286	5.01 ab
	25	5790	210	3.65 b
Field	2.5	5725	275	4.83 ab
	5	5714	286	5.01 ab
	25	5972	28	0.47 c

[1]Means followed by the same letter do not differ significantly at the 5% level by Tukey test.

RESUMO

Avaliou-se o efeito antiproliferativo de infusões de *Pluchea sagittalis* usando o teste de *Allium cepa*. Foram usadas infusões em três concentrações (2,5, 5 e 25g dm^{-3}) de folhas cultivadas em três ambientes (*in vitro*, sala de crescimento climatizada e em campo). Foram usados seis grupos de bulbos para cada um dos 8 tratamentos e o os índices mitóticos foram obtidos a partir de 6000 células por tratamento. Concluiu-se que a infusão de folhas de *P. sagittalis* cultivadas em campo possui grande atividade antiproliferativa, bem como o sistema de cultivo de plantas influencia o potencial antiproliferativo.

Palavras-chave: efeito antiproliferativo, quitoco, ciclo celular, planta medicinal, mutagenicidade.

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