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Antiproliferative effect of extracts of *Sida rhombifolia* L. (Malvaceae) on the *Allium cepa* cell cycle

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ABSTRACT: Field collected roots of four populations of *Sida rhombifolia* were used for preparing aqueous decoctions at two concentrations: 4g/L; and 16g/L. Afterwards, we used three groups of six onion (*Allium cepa*) bulbs for testing each population. Slides were made with all bulbs through the smashing technique. Cells in all phases of the cell cycle of *A. cepa* were analyzed. The mitotic index (% of cells in mitosis) was calculated, and the statistical analysis through the $\chi 2$ test was carried out at 5% probability. The results showed that the aqueous extracts of *S. rhombifolia* have antiproliferative activity at high concentrations. Practically no chromosomal aberrations were induced by treatments.

Medicinal plants are widely used in Brazilian folk medicine for treating human illnesses, mainly as infusions or decoctions (teas). However, their uncontrolled use may cause more damages than benefits to public health. According to Teixeira *et al.,* (2003) the use of medicinal plants is a particularly common practice in developing countries, and Vicentini *et al.* (2001) reported that medicinal plants may contain toxic substances or cause mutagenic effects; on the other hand, the consumption of teas can suppress the effects of mutagenic agents which are acting upon humans (Vicentini *et al.,* 2001).

Sida rhombifolia L. (known as guanxuma or broom) is native to the American continent and is found throughout Brazil (Lorenzi and Matos, 2008). It may be considered as a pest for agriculture, but its teas are popularly used in Brazilian folk medicine. Leaves are used for its mucilage and pleasant taste as a remedy for diarrhea. Australian aborigines also use a root decoction for similar purposes, and eat the raw roots to relieve 'indigestion' (Sens, 2002). In India, a root infusion is used for treating rheumatism. Also, several other uses are mentioned by Lorenzi and Matos (2008). However, neither the efficacy nor the safety of these uses have been tested in the laboratory.

This study was aimed at evaluating the antiproliferative effect of liquid extracts of *S. rhombifolia* on the *A. cepa* cell cycle.

Roots were collected from four populations in the Region of the Central Depression: population 1, Camobi/Santa Maria, Km 10/Highway (29°42'20" S; 53°44'05" W); Population 2, Santa Maria-Rosário (29°42'30" S; 53°52'02" W); Population 3, Silveira Martins (29°38'57" S; 53°34'55" W), and Population 4, UFSM/Santa Maria (29°43'00" S; 53°43'45" W).

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The plant roots were chopped and decocted for 10 min in boiling water. Then the decoctions extracts were filtered and chilled to room temperature. Two concentrations were used 4 and 16 g/L (4 g/L approximates the one used in folk medicine).The decoctions from populations 2 and 3 were stored in the refrigerator for 42 days before experimental use.

Onion bulbs (3 groups of 6 onions) bulbs were allowed to root in water, and treatments were applied when rootlets were 5-10mm long for 24 h. Afterwards, the rootlet tips were collected and fixed in ethanol:acetic acid (3:1) for 24 hours, and then kept in 70% ethanol in the refrigerator until observations.

The number of cells in interphase and in each phase of mitosis was determined as previously described by Tedesco and Laughinghouse IV (2012), the percent of cells in mitosis (mitotic index) was calculated and differences in the mitotic index were evaluated with the χ^2 test (at $P<0.05$ level, Bioestat 3.0 program). The effect of S. rhombifolia extracts on the number of cells in interphase and in each phase of mitosis is shown in Table 1. With only some exceptions, extracts from the four studied populations decreased the number of cells in prophase, metaphase, anaphase and telophase, in a dose-dependent manner.

Table 2 shows that extracts of *S. rhombifolia* from all the studied populations produced a statistically significant decrease (χ^2 test) in the mitotic index (MI), at the concentration popularly used for human use of these decoctions. With one exception (population 2), a 4-fold increase in concentration produced a further decrease.

It should be noted that only a very small number (2 or less among 6000 studied cells per treatment) showed chromosomal aberrations (not shown in Table 2). It is concluded that root decoctions of *S. rhombifolia* have a clear antiproliferative effect in the *A. cepa* test, but that they do not show genotoxic activity in terms of chromosomal aberrations, and that its use in folk medicine may be considered safe.

Antiproliferative activity in the *A. cepa* test has been shown for leaf and bark. extracts of *Luehea divaricata* Martius (Frescura *et al.,* 2012), inflorescence extracts of *Achyrocline satureioides* (Lam.) DC (Fachinetto *et al.,* 2007), and leaf extracts of *Pterocaulon polystachyum* DC (Knoll *et al.,* 2006) and *S. rhombifolia* (Islam *et al.* 2003), but no studies on root extracts have been made before the current paper.

TABLE 1

Number of cells in cellular cycle (interphase, prophase, metaphase, anaphase and telophase) in onion root tips treated with decoctions of *Sida rhombifolia*

		Number of cells in cell cycle phases				
Populations	**Treatments**	**Interphase**	**Prophase**	**Metaphase**	**Anaphase**	**Telophase**
	Control	4685	737	263	204	111
1	[4 g/L]	5803	95	36	45	21
	[16 g/L]	5888	56	32	15	9
	Control	5564	268	68	89	19
2	[4 g/L]	5684	170	63	77	6
	[16 g/L]	5578	211	86	121	4
	Control	5704	158	36	94	8
3	[4 g/L]	5909	73	8	7	3
	[16 g/L]	5932	39	13	15	1
	Control	4955	433	235	294	83
4	[4 g/L]	5898	58	28	18	4
	[16 g/L]	5944	20	21	15	0

TABLE 2

Mitotic indexes in onion root tips treated with *Sida rhombifolia* decoctions

Populations	Treatments	Total number of cells	Dividing cells	Mitotic index (%)
1	Control	6000	1315	21.9 a
	[4 g/L]	6000	197	3.2 b
	[16 g/L]	6000	112	1.8 c
2	Control	6000	436	7.3 a
	[4 g/L]	6000	316	5.3 b
	[16 g/L]	6000	422	7.0 a
3	Control	6000	296	4.8 a
	[4 g/L]	6000	91	1.4 b
	[16 g/L]	6000	68	1.0 c
4	Control	6000	1045	17.3 a
	[4 g/L]	6000	102	1.7 b
	[16 g/L]	6000	56	0.8 b

Indexes followed by the same letter did not differ significantly ($P<0.05$, χ^2 test).

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