



Citation: Guadalupe Velázquez-Vázquez, Beatriz Pérez-Armendáriz, Verónica Rodríguez Soria, Anabella Handal-Silva, Luis Daniel Ortega (2022) Genotoxicity and cytotoxicity of *Sambucus canadensis* ethanol extract in meristem cells of *Allium sativum*. *Caryologia* 75(1): 99-107. doi: 10.36253/caryologia-1307

Received: May 6, 2021

Accepted: December 17, 2021

Published: July 6, 2022

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

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Genotoxicity and cytotoxicity of *Sambucus canadensis* ethanol extract in meristem cells of *Allium sativum*

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Abstract. *Sambucus canadensis* is used in traditional medicine mainly in indigenous communities as an anti-inflammatory, antiviral, to treat cough, fever and other ailments, however, its use must be validated on scientific bases. The aim of this study was to evaluate the genotoxic and cytotoxic effect of the ethanol extract of *Sambucus canadensis* in meristem cells of *Allium sativum* with 5 treatments at concentrations of 125, 250, 500, 1000 and 1500 mg/L. Two thousand cells were counted per treatment; the mitotic index (MI) and nuclear abnormalities (NA) were evaluated. Data were analyzed using variance analysis (ANOVA) and Chi square (X^2) ($p < 0.05$). Root growth was found to be inhibited based on the concentration with statistically significant differences ($p < 0.05$). As the dose and exposure time of the ethanol extract increased, the MI decreased. The NA increased at the highest concentrations of 500, 1000 and 1500 mg/L and these differences were statistically significant compared to the control ($p = 0.001$). With the results obtained, it can be shown that the species has antiproliferative effects and genotoxic activity on the *Allium sativum* cell cycle, which can be extrapolated to other types of eukaryotic cells. Therefore, despite being a plant with health benefits, moderate use and low concentrations are recommended to avoid harmful effects.

Keywords: traditional medicine, chromosomal aberrations, biomodel, *Allium sativum*, genotoxicity, elderberry, plant extract.

INTRODUCTION

Medicinal plants are used by rural and urban populations in the treatment of numerous diseases (Chabán *et al.* 2019; Trap *et al.* 2020). In various parts of the world, they are the only source of medical care, mainly due to economic and geographical factors, customs and traditions (Marcotullio *et al.* 2018; Tedesco *et al.* 2017; Ullah *et al.* 2013). According to the World Health Organization (WHO), about 80% of the world's population uses herbal remedies as primary health care. The use of plants to treat dis-

eases is still based on empirical knowledge, although they have been considered low risk compared to other synthetic drugs (De Smet 2007; Monroy *et al.* 2005, Newman and Cragg 2016). The scientific information available for most medicinal plants is still insufficient to guarantee their safe and efficient use (Moore *et al.* 2020; Pastori *et al.* 2013). Various studies have indicated the importance of evaluating their safety (Ganjhu *et al.* 2015; Huang *et al.* 2015; Neira *et al.* 2018; Palatini and Komarnytsky 2019; Soliman 2010; Sousa *et al.* 2011, Vazirian *et al.* 2018) due to possible risks associated with their components, which may be potentially toxic, mutagenic, carcinogenic or teratogenic (Abdelmigid 2013; Bratu *et al.* 2012; Prasansuklab *et al.* 2020). Among the medicinal plants with high curative potential is *Sambucus canadensis* (L.) Bolli. A native of Mexico belonging to the *Adoxaceae* family, it is commonly known as elderberry. This species has been used medicinally in indigenous communities as a bactericide, anti-inflammatory, against flu, cough, dysentery, fever, as well as in uses related to rituals for pregnant women (Álvarez-Quiroz *et al.* 2017; Lee and Finn 2007; Sánchez-González *et al.* 2008; Wu *et al.* 2004). Its antimicrobial, antiviral, antioxidant and chemopreventive activities, among others (Sidor *et al.* 2014; Tedesco *et al.* 2017; Thole *et al.* 2006) have been associated with the components present in the species such as triterpenes, tannins and various types of flavonoids such as anthocyanins (Abdelmigid 2013; Ozgen 2010; Vujosevic *et al.* 2004), however, the presence of these compounds could also cause harmful effects such as nausea, vomiting and diarrhea, such as in the case of *Sambucus nigra* species, whose consumption in pregnant and lactating women, as well as in children and teenagers under 18 years of age, should be avoided (EMA/HMPC European Medicines Agency 2012). Information on toxicology, cytotoxicity and genotoxicity of *Sambucus canadensis* leaves is limited (Knudsen and Kaack 2015; Lee and Finn 2007; Schmitzer *et al.* 2012). It is important therefore to carry out studies to evaluate chromosomal damage and alterations of the mitotic cycle. According to Hister *et al.* (2017); Nefic *et al.* (2013); Pinho *et al.* (2010); Souza *et al.* (2010); Tedesco *et al.* (2015), the *Allium sp.* biomodel is a widely used, efficient, fast, low-cost method, with extrapolatable results since animal and plant chromosomes have similar structures. The aim of this study was to evaluate the cytotoxic and genotoxic effect of *Sambucus canadensis* on meristem cells of *Allium sativum*.

MATERIALS AND METHODS

Plant material

The *Sambucus canadensis* (L.) Bolli. plant was collected in San Miguel Eloxochitlan in the Sierra Negra zone of Puebla, Mexico, coordinates 18°30'32"N and 96°55'22"W. The plant material was identified using taxonomic techniques and one specimen was deposited in the Arboretum of the University of Puebla Botanic Garden (JB-BUAP) with the ID: 83771.

Preparation of Sambucus canadensis extract

The leaves of *Sambucus canadensis* (L.) Bolli. were used. The extract was obtained by macerating 750g the dry leaves of seven plants with 4L of 96% ethanol with double filtering. The extracts were vacuum filtered with Whatman No. 4 paper, the supernatant was concentrated on a Buchi® rotary vapor under reduced pressure at 35 ± 15 °C and the ethanol extract evaporated *in vacuo*. Later, different concentrations of the extract were made, specifically, 125 mg/L, 250 mg/L, 500 mg/L, 1000 mg/L and 1500 mg/L. Phytochemical tests were carried out for the qualitative identification of the different metabolite groups, each test was performed in triplicate (Carvajal *et al.* 2009; Patil and Bhise 2015). Fourier-transform mid-infrared spectroscopy (FTIR) from 4000 to 600 cm⁻¹ was used to obtain information about the functional groups present in the plant using a Bruker spectrometer at a resolution of 4 cm⁻¹. The analyses were done in the High Technology Service Center (CESAT-UPAEP).

Allium sativum bioassay

Meristem cells from the roots of *A. sativum* were used to evaluate the nuclear abnormalities (NA) and the mitotic index (MI) in the concentrations (125, 250, 500, 1000, 1500 mg/L); water was used as a control. Five repetitions were performed on each concentration with bulbs of uniform size (3 cm in diameter). The control bulbs were kept in water.

The other bulbs were transferred to the different concentrations for 120 hours. At the end of exposure, the length of the roots and stem were measured and examined to detect visible morphological anomalies: changes in consistency and root color and the presence of hooks or twists in the roots as a sign of general toxicity (Çelik and Aslantürk 2010). Subsequently, the meristem zone of the garlic roots (2 mm) was cut and placed on a slide, hydrolyzed in 1N HCl for 10 minutes,

then washed with distilled water, and stained with acetic orcein. The slides were fixed with the squash method sealing the edges with resin. The samples were analyzed with a Leica DM1000 LED fluorescence optical microscope with a Jenoptik ProGres C10 digital camera. Some 2000 meristem cells were counted for each treatment. In the stages of mitosis (interphase, prophase, metaphase, anaphase and telophase), the cellular alterations were counted: chromosomal breakage, bridges, lagging chromosomes, strays, among others. The values obtained were used to calculate the mitotic indices (MI) and the percentage of cellular alterations (CA) with the following formulae:

$$MI = \text{Number of cells in mitosis} \div \text{Total cells} \times 100$$

$$CA = \text{Number of cells with abnormal chromosomes} \div \text{Total cells} \times 100$$

The results of the number of roots and length of roots and stem, and the mitotic index were analyzed with the ANOVA (Bonciu *et al.*, 2018). The differences were evaluated with Dunnett's post hoc test. The nuclear abnormalities were evaluated with the Chi-squared test (X^2) using the Minitab 8.1 statistics program. Values of $p < 0.05$ were considered significant differences.

RESULTS

The phytochemical tests of the *Sambucus canadensis* extract revealed the presence of alkaloids, flavonoids, saponins and tannins. The infrared spectrum tests (FTIR) on the extract showed different frequencies of stretching and bending; the stretching frequencies of the O-H bond at 3350 cm^{-1} is associated with phenol groups; the involvement in hydrogen bonding produces a widening of the band. The C-H bond stretching vibrations corresponding to methyl and methylene groups appear in the $3000\text{--}2850 \text{ cm}^{-1}$ range and the bands in the fingerprint region are due to the bending vibrations at 1386 cm^{-1} for methyl and 716 cm^{-1} for ethyl: the stretching vibrations of the carbonyl bond, C=O, appears in the $1750\text{--}1680 \text{ cm}^{-1}$ range related to the presence of flavonoids. Similarly, a conjugated double bond C=C of the aromatic rings appears in the $1600\text{--}1450 \text{ cm}^{-1}$ range, characteristic of the basic structure of flavonoids (Figure 1).

The length of *Allium sativum* roots and stem at the 1000 and 1500 mg/L concentrations were 7.66 mm and 0.72 mm, respectively, showing statistically significant differences compared to the control ($p = 0.000$). There

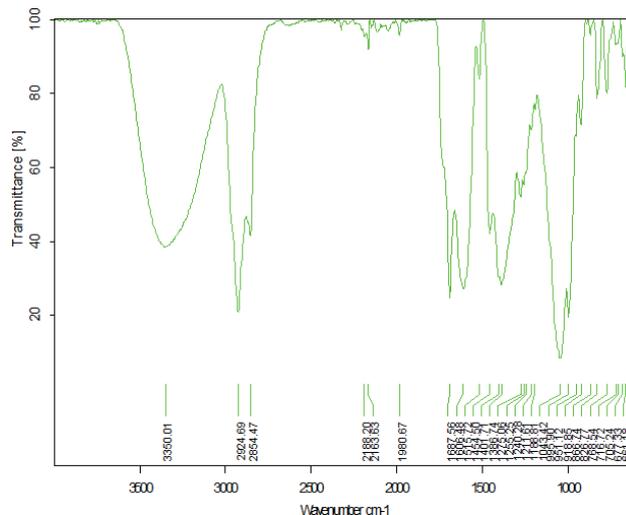


Figure 1. FTIR spectra of *Sambucus canadensis* in the range of 4000 to 600 cm^{-1} .

were no significant differences with the other treatments ($p > 0.05$). The stem length at a concentration of 1500 mg/L (2.36 mm) showed a statistically significant difference compared to the stem length of the control group ($p = 0.000$). It can be seen that the average length and number of roots decreased depending on the concentration (Table 1). In terms of morphology, at concentrations of 125 and 250 mg/L no differences were observed compared to the control, however, at concentrations of 500 and 1000 mg/L the roots appeared yellow, at 1500 mg/L the sparse roots were brown and stiff. Likewise, effects on the stem such as twisting and color change were observed, mainly at concentrations of 1000 and 1500 mg/L.

Regarding the results of the mitotic index, Table 2 shows a decrease in the MI as concentration increases; the number of dividing cells (prophase, metaphase, anaphase and telophase) differed between concentrations, however, at 125, 250 and 500 mg/L there were no significant differences compared to the control ($p > 0.05$). At a concentration of 1500 mg/L a statistically significant difference was observed in the mitotic index compared to the control.

The nuclear abnormalities in *Allium sativum* are shown in Table 3. Concentrations of 500, 1000 and 1500 mg/L of the ethanol extract had the highest number, concentrations of 125 and 250 mg/L a lesser amount. The number of cells in mitosis with anomalies was related to the increase in concentration. Figure 2 shows abnormalities such as breakage, chromosome loss, bridges, chromosomes with inactivated centromere, among others.

Table 1. Number and length of roots, and stem length of *A. sativum* exposed to the ethanol extract of *Sambucus canadensis*.

Treatment mg/L	Number of roots (x) (δ)	Length of roots (x) (δ)	Stems length (x) (δ)
125	13.2 \pm 2.66	15.82 \pm 7.24	25.52 \pm 17.54
250	9.84 \pm 4.94	11.47 \pm 6.12	18.76 \pm 10.81
500	11.20 \pm 6.81	19.76 \pm 12.95	20.8 \pm 12.62
1000	7.36 \pm 3.68	8.66 \pm 10.32*	17.6 \pm 8.43
1500	0.92 \pm 0.90 *	0.72 \pm 0.42 *	2.36 \pm 0.46*
Control	13.4 \pm 7.96	25.84 \pm 19.79	36.3 \pm 44.25

Values are mean \pm S.E, One way ANOVA (*) are not significantly different $p < 0.05$.

DISCUSSION

The test with *Allium spp.* is a suitable biomodel for identifying the cytotoxic and genotoxic effects of different plants (Bagatini *et al.* 2007; Lubini *et al.* 2008; Trapp *et al.* 2020). The study of raw extracts is important since

traditional medicine uses part of the plant structure (leaves, stem, root) or the whole plant, without separating its components. Furthermore, it has been shown that different bioactive compounds act synergistically (Tallarida 2011) and that a combination of compounds exhibits a greater effect than individual compounds, suggesting that the effects of some plants are the result of the interaction of their components (Lamy *et al.* 2018).

In this study, the qualitative analyses (phytochemical tests; FTIR) of the plant showed the presence of alkaloids, tannins, saponins and flavonoids. The spectroscopy used provides important information about functional groups as well as being an accessible and useful technique in the chemical and structural analysis of plants (Günzler and Gremlich 2002; Heredia-Guerrero *et al.* 2014). The functional group associated with the signals reported in the evaluated spectra (FTIR) is flavonoids, which present inhibitory activity against diverse fungi and bacteria species. The metabolites reported (alkaloids, tannins, saponins, flavonoids) showed antimicrobial, antioxidant and antiviral activ-

Table 2. *Allium sativum* merismatic cell numbers in the different cell cycle phases, and index mitotic extract of *Sambucus canadensis*.

Treatment mg/L	Interphase	Prophase	Metaphase	Anaphase	Telophase	Cells in division	Mitotic Index (%)
Control	3831	1988	81	59	41	2169	36,1
125	4020	1840	66	39	35	1980	33
250	4356	1571	29	23	21	1644	27,4
500	4306	1580	58	34	32	1704	14,3
1000	4558	1330	36	15	21	1402*	12,0
1500	5398	556	17	13	16	602*	5,03

* $p < 0.05$ in One Way ANOVA.

Table 3. Cellular abnormalities observed in *Allium sativum* exposed to the ethanolic extract of *Sambucus canadensis*.

	Treatments mg/L					
	Control	125	250	500	1000	1500
Number of cells in division	2169	1980	1644	1704	1442	602
Bridges	-	-	1	4	37	25
Chromosome fragments	-	-	2	-	4	5
Binucleate	2	3	24	99	40	30
Chromosome lagging and disoriented	2	1	1	7	7	4
Sticky chromosome	-	1	-	1	5	2
Vagrant chromosome	-	-	-	2	3	4
Trinucleated	-	-	-	10	5	20
Total cells aberrations	4a	5a	28b	123b	101b	90b
Cells aberration (%)	0,2	0,3	1,7*	7,2*	7,0b	15,0b

*The chi-square test. Significant difference $p < 0.05$.

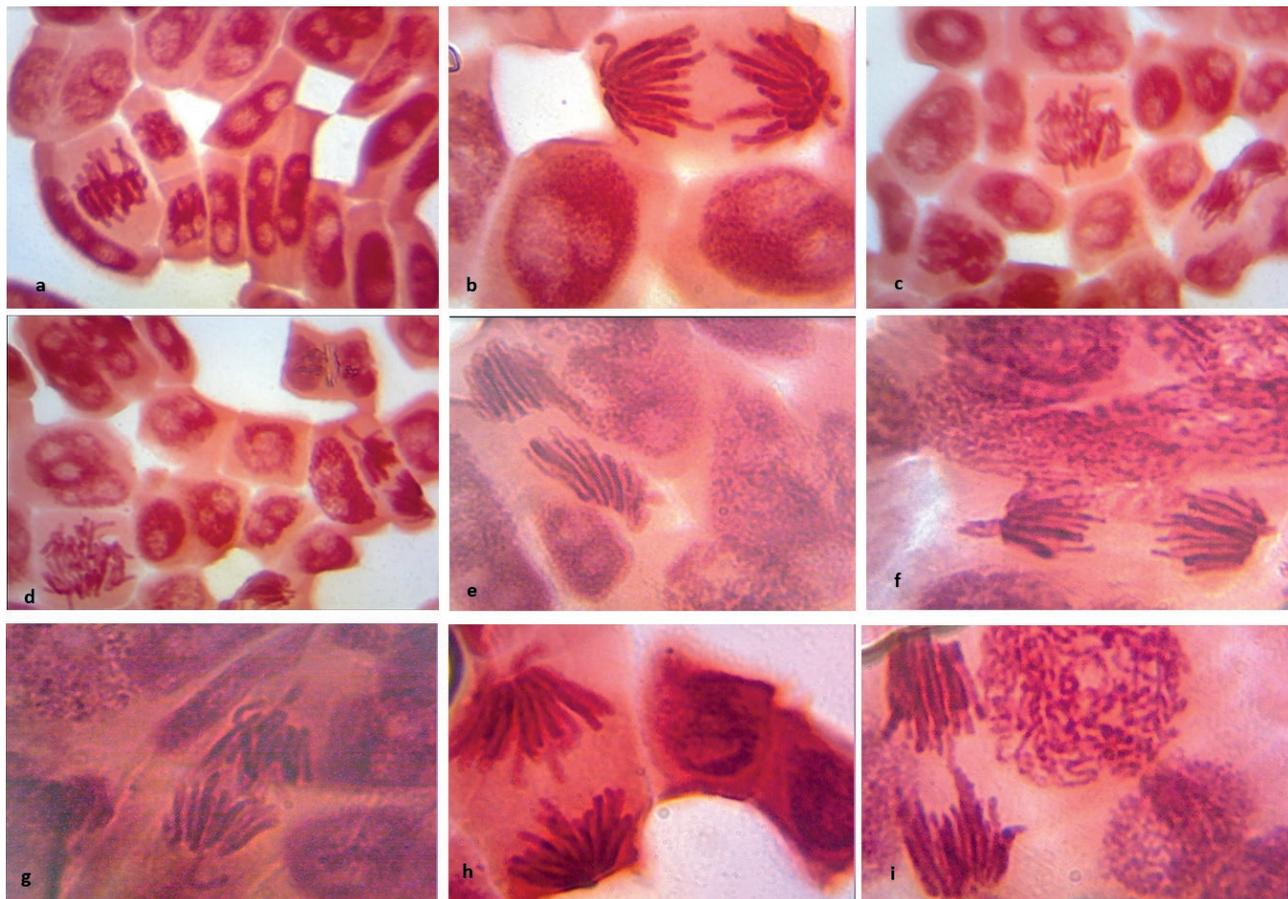


Figure 2. *Allium sativum* cells exposed to the ethanolic extract of *Sambucus canadensis* a) sticky chromosome b) bridges c) sticky and lagging chromosome with bridges d) lagging and sticky chromosome e) abnormal anaphase f) lagging chromosome g) vagrant chromosome and lagging chromosome, h) bridge and vagrant chromosome, i) bridges.

ity, among others. Flavonoids in particular exhibit important pharmacological activities, in addition to being effective in chemoprevention and chemotherapy (Paduch *et al.* 2007; Perveen 2018). The evaluation of the *S. canadensis* extract with the *Allium sativum* test allowed us to determine the effects on root and stem growth as well as morphology; the highest concentrations, 1000 and 1500 mg/L, significantly inhibited root and stem growth compared to the control. The mitotic index (MI) decreased significantly as the concentration of *Sambucus canadensis* increased, matching the results reported for other species of *Sambucus sp.* (Tedesco *et al.* 2017; Thole 2006). Other authors have reported that plant extracts such as *P. leiocarpa* and *P. myriantha* (Lubini *et al.* 2008), *Campomanesia xanthocarpa* (Pastori *et al.* 2013), *Vernonanthura polyanthes* (Almeida *et al.* 2020), *Amaranthus spinosus* (Prajitha and Thoppil, 2016), *Achyrocline satureioides* (Fachinetto *et al.* 2007), *Luehea divaricata* (Frescura *et al.* 2012) caused

a reduction in the mitotic index when increasing the concentration, which may be an indication of antiproliferative activity such as that reported by Bagatini *et al.* (2009), Knoll *et al.* (2006). The results obtained in this study may be associated with the plant components; in this sense, the flavonoids found in the FTIR analysis may inhibit or stimulate the cellular cycle. Tedesco *et al.* (2017) found that *Sambucus australis* has flavonoids such as rutin, kaempferol and quercetin, among others, to which different pharmacological effects have been attributed, including antiproliferative and anticancer action. One study developed by Lee and Finn, (2007) reported that *Sambucus canadensis* presents a high quantity of anthocyanins and polyphenols which have a potent antioxidant effect, perhaps also explaining the inhibition of cellular division in *Allium sativum*. In the same way, the phenolic components of the species have been associated with a more potent anticancer activity than *Sambucus nigra* (Thole *et al.* 2006).

The main chromosomal aberrations found in this study include the formation of bridges, which, according to Türkoğlu (2007), are produced due to the fusion of chromosomes or chromatids as a result of chromosomal stickiness or due to unequal translocation. Lagging chromosomes moving to both sides of the poles without being fused by the spindle apparatus can also induce bridges. Another aberration found in the results of this investigation were sticky chromosomes formed by the free movement of chromosomes, which can produce chromosomal breakage and may lead to the loss of genetic material (Dutta *et al.* 2018). Stray chromosomes advance ahead of the chromosome group towards the poles resulting in an unequal distribution of chromosomes in daughter cells (Sondhi *et al.* 2018).

Similarly, Fachinnetto and Tedesco (2009) attribute various chromosomal anomalies, bridges, binucleated cells, among others, to the components of the plants. Along these lines, Bagatini *et al.* (2009); Toloza *et al.* (2006), indicate that the genotoxic and antiproliferative activity presented by some plant extracts are the result of the interactions of their different chemical components. In this regard, Amado *et al.* (2020) reported that the *Smilax brasiliensis* extract and the rutin and quercetin fractions, which have also been found in the species *Sambucus sp.* cause genotoxic effects. It has been suggested that, if extracts cause damage to plant cell chromosomes, they may also be potentially harmful for mammalian cell chromosomes (Feretti *et al.* 2007).

According to the results, no abnormalities in the *A. sativum* root were found at low concentrations of 125 mg/L and 250 mg/L. However, in concentrations of 500, 1000 and 1500 mg/L, a considerable number of alterations, such as bridges, chromosome breaks and strays were found, suggesting that the extract presents a genotoxic effect at high concentrations. The results match those reported by Bratu (2012) which indicate that at low concentrations the species *Sambucus nigra* presents no mutagenic effects. According to Ifeoluwa *et al.* (2013) and Sabini *et al.* (2011), some plants induce cytotoxicity but not mutagenic effects. Generally speaking, the frequency of aberrations increases significantly as the concentration increases, suggesting that *Sambucus canadensis* leaf extract presents clastogenic effects, which agrees with the reports of Bidau *et al.* (2004); Çelik and Aslantürk (2010) and Mattana *et al.* (2014) on the effects of herbal plants.

Sambucus canadensis is a plant that should be used with caution not only because it is used for curing diseases but also because it is used by pregnant women (Velázquez-Vázquez *et al.* 2019) and could be harmful to their health. The use of plants before and after pregnan-

cy may cause conditions from vomiting, infection and gastrointestinal problems to placental retention, uterine hypotonia, cervical tear, miscarriage, uterine bleeding and others. Since few studies exist on the safety and efficacy of the use of herbal plants during pregnancy, a situation which exposes both the mother and the fetus, it is recommended that they are not used during pregnancy unless such use is supported by scientific studies which validate their safety (Ahmed *et al.* 2017; Frawley *et al.* 2015; Hall *et al.* 2011; Illamola *et al.* 2020; Nergard *et al.* 2015).

CONCLUSION

The results of this study suggest that the ethanol extract from *Sambucus canadensis* induces antiproliferative effects. It was also found that in concentrations higher than 500 mg/L the extract affects root growth, cellular division and chromosomal changes in the cells of *Allium sativum*. It is important to know the effects of plants that are used as the primary source of medical care in order to contribute to the regulation of their use and consumption as an important measure for protecting human health.

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