Cytotoxicity, genotoxicity and antiprofilerative effect of *Costus Scaber* Ruiz & Pav (Costaceae): a contribution to folk medicine, oncology and genetic improvement

Citotoxicidade, genotoxicidade e efeito antiprofilerativo de *Costus scaber* Ruiz & Pav (Costaceae): uma contribuição para medicina popular, oncologia e melhoramento genético

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ABSTRACT

Costus scaber is an herbaceous species that has hard and cylindrical stems and can reach up to 80 cm in height. It has an inflorescence at the end of the stalk from 6 to 10 cm long, which is surrounded by scaly bracts. Due to this species interesting medicinal potential, the objective of this study was to analyze its cytotoxic and genotoxic effect. The cytogenotoxic and antiprofilerative effect was evaluated using the bioindicator *Allium cepa* via the direct and indirect method. Fresh Leaves of *C. scaber* were used for the infusion. Three different concentrations of infusions were elaborated, which were 2, 3 and 4 g/L^{-1} for each of the different methods, and were analyzed at 24, 48 and 72 hours. Five slides were made per treatment, with 300 cells per slide. Statistical analyses were performed in the R program. The cytotoxic, genotoxic and antiprofilerative effects were found in certain concentrations and at certain exposure periods, and it was also noted that, in general, as the concentration and exposure period increased, the mitotic index decreased and the antiprofilerative effect increased. Several chromosomal abnormalities were found in the infusions analyzed. Therefore, according to the results found, it can be concluded that *C. scaber* has great potential for genetic improvement of plants in the field of drug production mainly in the field of oncology.

Keywords: Chromosomal abnormalitie, Cytotoxicity, Genotoxicity.

RESUMO

Costus scaber é uma espécie herbácea que possui hastes duras e cilíndricas, podendo atingir até 80 cm de altura, apresenta inflorescência em espiga terminal de 6 a 10 cm, cercadas por brácteas escamosas. Devido possuir grande potencial medicinal, o objetivo deste estudo foi analisar o efeito citotóxico e genotóxico desta espécie. O efeito citogenotóxico e antiprofilerativo foi avaliado pelo bioindicador *Allium cepa*, pelo método direto e indireto. Foram utilizadas folhas frescas de *C. scaber* para a infusão. Três diferentes concentrações das infusões foram elaboradas, sendo: 2, 3 e 4 g/L^{-1} para os diferentes métodos. Foram analisados nos tempos de 24, 48 e 72 horas. Confeccionadas 5 lâminas por tratamento e contabilizado 300 células por lâmina. As análises estatísticas foram realizadas no Programa R. Foram encontrados efeitos citotóxicos, genotóxicos e antiprofilerativo em determinadas concentrações e tempos. Notou-se ainda que de maneira geral conforme aumentou a concentração e o tempo, menor foi o Índice Mitótico e maior o efeito antiprofilerativo. Foram encontradas várias anormalidades cromossômicas nas infusões analisadas. Então de acordo com os resultados encontrados, pode-se concluir que *C. scaber* possui grande potencial para o melhoramento genético de plantas no quesito de produção de fármacos principalmente no ramo da oncologia.

Palavras-Chave: Anormalidades cromossômicas, Citotoxicidade, Genotoxicidade.

1 INTRODUCTION

Species of the genus *Costus* are perennial, cespitous plants, whose aerial part can reach from 1.0 to 2.0 meters in height. Some species are found almost all over Brazil, mainly in the Atlantic Forest and Amazon region. The leaves are most often spirally arranged, with an invaginative prolongation at the base forming an ochrea. Its terminal inflorescences have spiral bracts that are dense, imbricate, glabrous, and red in color (Lorenzi and Matos, 2008; Paes et al., 2013; Li et al., 2019).

Most species of the genus *Costus* have spiral branches with terminal inflorescences that produce only one flower per day (rarely two), in addition to an extended flowering period. Due to this, the species are widely used in ornamentation as cut flowers (Specht et al., 2001; Duarte et al., 2017).

In addition, species of the genus are used in Brazilian folk medicine, mainly in the Amazon region (Lorenzi and Matos, 2002; Santos et al., 2019). Passos (2019) affirms that, besides being used as an ornamental plant, the species *Costus scaber* Ruiz & Pav is widely used in folk medicine because it has secondary compounds in its rhizomes, stems, flowers and leaves, which can be used to treat various urinary problems and many problems related to water retention.

It is widely used in the treatment of amenorrhea, kidney stones, menstrual disorders, rheumatic pain, difficulty urinating, nephritis and urethritis. In addition, it is also used in food as an unconventional food plant; the leaves and flowers are used in the juices, jellies and salads (Martins et al., 2003; Menezes, 2007; Paes et al., 2013).

Due to the medicinal importance of *C. scaber*, it is necessary to perform a cytotoxic and genotoxic study of this species. For this type of study, the *Allium cepa* test is widely used, and this is most used among the main plant bioassays (Fiskesjo, 1985). The popularity of this assay is due to its cost-effectiveness, since it involves a simple methodology that requires minimal use of laboratory equipment. Its chromosomal number is (2n = 16), that is, there are few chromosomes with good size (8–16 µm), which are characteristics that facilitate microscopic analysis (Bolle et al., 2004; Ozkara et al., 2015).

C. scaber is not on the list of herbal medicines regulated by ANVISA, the Brazilian equivalent of the FDA. For this reason, some information about its medicinal use may not be entirely correct, such as in the preparation of its infusion, where only its popular use was found in the literature, however without scientific proof (Nascimento and Vieira, 2013).

Thus, this study aimed to evaluate the cytogenotoxicity and antiprofilerative effect of four species of the genus *Costus* by means of the *Allium cepa* test using the direct and indirect method with different concentrations and exposure periods, in order to provide information for the population that uses these plants and also for genetic improvement for drug extraction.

2 MATERIAL AND METHODS

Study area

This study was developed at the State University of Mato Grosso, Alta Floresta, Mato Grosso Campus, in the Cytogenetics and Culture of Plant tissue Laboratory.

Sampling of plant material

The sampling was performed in the city of Paranaíta in the state of Mato Grosso, Brazil (S 9°41' 57" W 56°27' 16"). After sampling, all material was sent to the Herbarium of Santarém (HSTM) at the Universidade Federal do Oeste do Pará (UFOPA) for registration and identification (collection number 302 and HSTM 12520).

Methodological Procedures

For the experiment, three different concentrations of fresh leaves in infusion (2 g/L^{-1}, 3 g/L^{-1} and 4 g/L^{-1}) from the species *C. scaber* were evaluated.

In the preparation of the infusions, the method of infusion employed was as follows: boiling water for a period of ten minutes, and after this period, cooling to room temperature. Subsequently, the infusions were placed in disposable cups with rooted onion bulbs of an ideal size (approximately 1 cm) for the cytogenotoxicity test, (indirect method). The direct method was also carried out, in which the roots of the onions were germinated in direct contact with the infusions.

For both methods each concentration received five onion bulbs, including positive control (acetone 1%) and negative control (distilled water). The treatments consisted of three concentrations and three exposure periods; 24 hrs, 48 hrs and 72 hrs. Controls were used for means of comparison. There was a daily substitution of infusions and collection of roots according to the periods previously mentioned. At 72 hours, the treatments in the direct method, the size of five roots of each bulb were measured before being collected.

After being exposed to the different treatments, the root meristems were collected and fixed in fixative solution composed of methanol: acetic acid (3:1) with the realization of three consecutive changes of fixative and, subsequently, conditioned under refrigeration for further analysis.

Slide preparation

For the preparation of the slides, the roots were subjected to a washing process that consisted of 3 washes in distilled water with intervals of 10 minutes before and after being hydrolyzed in HCl 1N solution for 15 minutes.

For cytogenetic analysis, the crushing technique was used, whereby each radicle was placed on an optical microscopy slide and, with the aid of a scalpel, the root apex was removed and subsequently stained with aceto-orcein 2% and macerated with a glass stick, then covered with a slide cover. Excess dye was removed with filter paper (Guerra and Souza, 2002).

Material analysis

The observation of the slides was performed under an optical microscope under 400x magnification using the scanning technique. Five slides were made for each treatment, which totaled 300 cells per slide. Regular and irregular cells were analyzed in

interphase, prophase, metaphase, anaphase and telophase. The cells with anomalies were photographed with the use of 100x lens in a binocular photomic microscope (Leica ICC 50) coupled to a computer using the software LAZ EZ, v1.7.0.

Statistical analysis

The mitotic index was obtained by dividing the number of cells in mitosis (prophase + metaphase + anaphase + telophase) by the total number of cells (interphase + mitosis) and multiplying by 100.

For statistical analysis, the data were submitted to the analysis of variance and mean test using a Tukey test (5% significance) and the statistical program R (Ferreira et al., 2013).

3 RESULTS

Direct method

In the direct method, where the roots of onion bulbs were developed directly in contact with the infusion concentrations, in all treatments, only those left for 48 and 72 hours germinated and root germination for the period of 24 hours was not observed.

It is possible to observe in Table 1 that in the MI the concentration of 2 g/l^{-1} was the one that presented significant statistical differences between the three infusion concentrations of *C. scaber*. The period of 48 hours presented higher MI (17.13%) compared to the period of 72 hours (7.66%). However, it is possible to observe that in the other concentrations the period of 48 hours also presented similar results, since the MI had a higher percentage than the period of 72 hours. It is well-known that for this species the longer the exposure period of the roots in the tested solution the lower the MI. It can be noted that for the period of 72 hours there was a greater cytotoxic effect.

Table 1. Mitotic index (MI) and percentage of anomalies (PA) of *Costus scaber*, using the direct method under two different exposure periods.

Concentration	MI		PA	
	Exposure period			
	48h	72h	48h	72h
2 g/l^{-1}	17.13Ac	7.66Bb	1.53Abc	0.86Abc
3 g/l^{-1}	10.80Acd	6.26Ab	1.86Ab	1.60Ab
4 g/l^{-1}	7.53Ad	4.06Ab	2.93Ab	0.59Bbc
Negative control	44.26Aa	27.93Ba	0.20Ac	0.13Ac
Positive control	27.19Ab	23.46Aa	16.46Aa	22.26Aa
CV (%)	28.25		34.38	

*Means followed by the same lowercase letter in the column and uppercase in the row do not differ from each other when analyzed using the Tukey test at 5% probability. CV – Coefficient of variation

In regards to the percentage of anomalies (PA) (Table 1), it is observed that the longer the exposure period, the lower the anomaly index, This is due to the same process that occurred with the MI (the longer the exposure period, the less cell division), and this occurred with the division of abnormal cells as well. Therefore, it is noted that in the exposure period of 48 hours there was greater genotoxic effect.

Regarding the concentrations in Table 1, it is noted that in the MI for 3 and 4 g/l^{-1} (10.80% and 7.53%) there were no statistical differences for the period of 48 hours, but these differed from the concentration of 2 g/l^{-1} (17.13%). For the period of 72 hours, there were no statistical differences between concentrations. It is possible to observe that, both for the period of 48 hours and the period of 72 hours, the higher the tested concentration the lower the MI. However, all concentrations showed cytotoxic effect when compared with the negative control, but, in the concentration of 4 g/l^{-1} for both exposure periods, the MI was lower and presented a greater cytotoxic effect among the different concentrations.

When the concentrations were observed in relation to the PA (Table 1), it can be noted that there was division of abnormal cells in all the concentrations tested for both exposure periods. For the exposure period of 48 hours, the concentration of 4 g/l^{-1} showed greater genotoxic effect, while for the exposure period of 72 hours the concentration of 3 g/l^{-1} showed greater number of anomalies, indicating greater genotoxic effect.

When analyzing Table 2, it is possible to note that there were no statistical differences for the percentage of interfases (PI) between the different concentrations of *C. scaber* infusions. In the PI, the longer the exposure period, the greater the number of cells in interphase, i.e., the greater the antiprofilerative effect. In relation to the concentrations that showed the greatest antiprofilerative effect, 4 g/l^{-1} was the optimum, 34% for the exposure period of 48 hours and 23.40% for the exposure period of 72 hours.

Table 2. Percentage of *Costus scaber* interfaces (PI) using the direct method under two different exposure periods.

Concentration	PI	
	Exposure period	
	48h	72h
2 g/l^{-1}	81.33Aa	91.46Aa
3 g/l^{-1}	87.33Aa	92.13Aa
4 g/l^{-1}	89.53Aa	95.33Aa
Negative control	55.53Bb	71.93Ab
Positive control	56.33Ab	54.26Ac
CV (%)	11.22	

*Means followed by the same lowercase letter in the column and uppercase in the row do not differ from each other when analyzed using the Tukey test at 5% probability. CV – Coefficient of variation

Average root size

Regarding the average root size (Table 3), it was observed that there were significant statistical differences in the exposure periods of 48 and 72 hours, and the exposure period of 48 hours presented a lower mean. However, when comparing the concentrations for both exposure periods, no statistical differences were observed. Analyzing the three concentrations for the exposure period of 48 hours, it was observed that these were more toxic than the controls, including the positive control. For the exposure period of 72 hours, the positive control showed lower toxicity than the other concentrations, however there was no significant difference.

Table 3. Mean size of *Costus scaber* roots obtained from different exposure periods and three different infusion concentrations

Concentration	Exposure period	
	48h	72h
2 g/l^{-1}	5.80Bbc	14.92Aa
3 g/l^{-1}	3.00Bc	15.56Aa
4 g/l^{-1}	7.00Bbc	15.60Aa
Negative control	14.45Aa	16.36Aa
Positive control	10.29Aab	14.57Aa
CV (%)	30.14	

*Means followed by the same lowercase letter in the column and uppercase in the row do not differ from each other when analyzed using the Tukey test at 5% probability. CV – Coefficient of variation

Indirect method

According to Table 4, an analysis of the mitotic index (MI) obtained through infusions of *C. scaber*, shows us that for the concentration of 2 g/l^{-1} the highest MI occurred in the exposure period of 72 hours (8.80%), however it did not differ statistically from the other periods. For the concentration of 3 g/l^{-1}, the highest MI occurred in the exposure period of 24 hours (9.93%) which differed statistically from the other exposure periods. For the concentration of 4 g/l^{-1}, the period that presented the highest percentage of MI was 24 hours, however, this also did not differ statistically from the others.

For the percentage of anomalies (PA) (Table 4), it is noted that for the concentration of 2 g/l^{-1}, the exposure period of 72 hours presented higher PA (1.93%), but it did not differ statistically from the other periods. For 3 g/l^{-1}, the period of 48 hours had higher PA (4.13%), which did not differ statistically from the period of 72 hours (3.53%). And for 4 g/l^{-1}, the exposure periods of 24 and 72 hours presented the same PA (1.46%), but did not differ statistically from the period of 48 hours (0.73%).

Table 4. Mitotic index (MI) and percentage of anomalies (PA) of *Costus scaber* using the averages of the indirect method under three different exposure periods

Concentration	MI			PA		
	Exposure period					
	24h	48h	72h	24h	48h	72h
2 g/l^{-1}	5.53Ab	6.2Ab	8.80Aab	1.13Aa	1.33Ac	1.93Aac
3 g/l^{-1}	9.93Ab	6.79ABb	4.20Bb	1.06Ba	4.13Ab	3.53Aab
4 g/l^{-1}	11.53Ab	6.33Ab	9.39Aab	1.46Aa	0.73Ac	1.46Abc
Negative control	23.53Aa	16.2Bb	12.26Ba	0.20Aa	0Ac	0Ac
Positive control	7.93Ab	1.79Bb	9.93Aab	1.59Ca	7.53Aa	4.06Ba
CV (%)	38.98			62.19		

*Means followed by the same lowercase letter in the column and uppercase in the row do not differ from each other when analyzed using the Tukey test at 5% probability. CV – Coefficient of variation

When analyzing the MI in the concentrations of *C. scaber* for the exposure periods of 24 and 48 hours, it is noted that the concentration of 2 g/l^{-1} presented greater cytotoxicity than the other concentrations in these periods, while for the period of 72 hours the concentration with greater cytotoxic effect was that of 3 g/l^{-1}. However, all concentrations in all periods had a cytotoxic effect when compared with negative control (Table 4). Regarding the PA, analyzing the concentrations, it is observed that for the exposure period of 24 hours the concentration 4 g/l^{-1} showed greater genotoxic effect, however the percentage did not differ statistically from the other concentrations. For the exposure periods of 48 and 72 hours, the concentration of 3 g/l^{-1} showed greater genotoxic effect, statistically differing from the other concentrations.

According to Table 5, in relation to the percentage of interfases (PI), it can be noted that the results were very close in terms of comparison of exposure periods and concentrations and there were also no significant differences between the exposure periods.

Table 5. Percentage of *Costus scaber* interfaces (PI) using the indirect method under two different exposure periods.

Concentration	PI		
	Exposure period		
	24h	48h	72h
2 g/l^{-1}	93.33Aa	92.46Aa	89.26Aa
3 g/l^{-1}	89.0Aa	89.06Aab	92.26Aa
4 g/l^{-1}	87.0Aa	92.93Aa	89.13Aa
Negative control	76.26Bb	83.80Ab	87.73Aa
Positive control	90.46Aa	90.66Aab	85.99Aa
CV (%)	5.00		

*Means followed by the same lowercase letter in the column and uppercase in the row do not differ from each other when analyzed using the Tukey test at 5% probability. CV – Coefficient of variation

An analysis of the exposure period of 24 hours demonstrates that there were no significant differences between the concentrations, however the concentration of 2 g/l^{-1}

obtained a higher percentage (93.33%), which indicates that there was an antiprofiletrative effect of 17.07%. For the exposure period of 48 hours, the concentrations of 2 and 4 g/l^{-1} presented no significant differences between them, though they differed from the concentration of 3 g/l^{-1}, but the concentration of 4 g/l^{-1} presented a higher PI, which indicates that there was a greater antiprofilerative effect (9.93%). For the exposure period of 72 hours, there were also no significant differences, but the concentration of 3 g/l^{-1} presented a higher PA (92.26%) which indicates that there was an antiprofilerative effect of 4.53%.

To verify the genotoxic action of *C. scaber*, the mitotic cycle (MI) and interphase anomalies were analyzed. Several abnormalities were found, of which the main ones were anaphase with poles in two blocks, metaphase with ring chromosome, telophase with isolated chromosomes and presence of micronucleus, anaphase with chromosomal duplication, anaphase with chromosome lag, anaphase with chromosome bridge, interphase with extended nucleus and prophase with ring chromosome (Figure 1). The most common abnormalities were lagging chromosomes and isolated chromosomes (direct method) and, for the indirect method, the abnormality most frequently found was that of extended nucleus (Table 6).

Figure 1. Chromosomal abnormalities found in infusions of *C. scaber*: A) Anaphase with poles in two blocks; B) Metaphase with ring chromosome; C) Telophase with isolated chromosomes and the presence of micronucleus; D) Telophase with micronucleus; E) Anaphase with chromosomal duplication in one of the blocks and isolated chromosomes; F) Anaphase with lagging chromosomes and isolated chromosomes; G) Anaphase with chromosome bridge and lagging chromosomes; H) Anaphase with isolated chromosomes and C chromosomes; I) Metaphase with ring chromosome; J) Metaphase with chromosome in C; K) M with ring chromosome; L) Interface with extended core; M) Prophase with ring chromosome; N) Anaphase with chromosome bridge, lagging chromosomes and isolated chromosomes. Image bar = 10 µm.

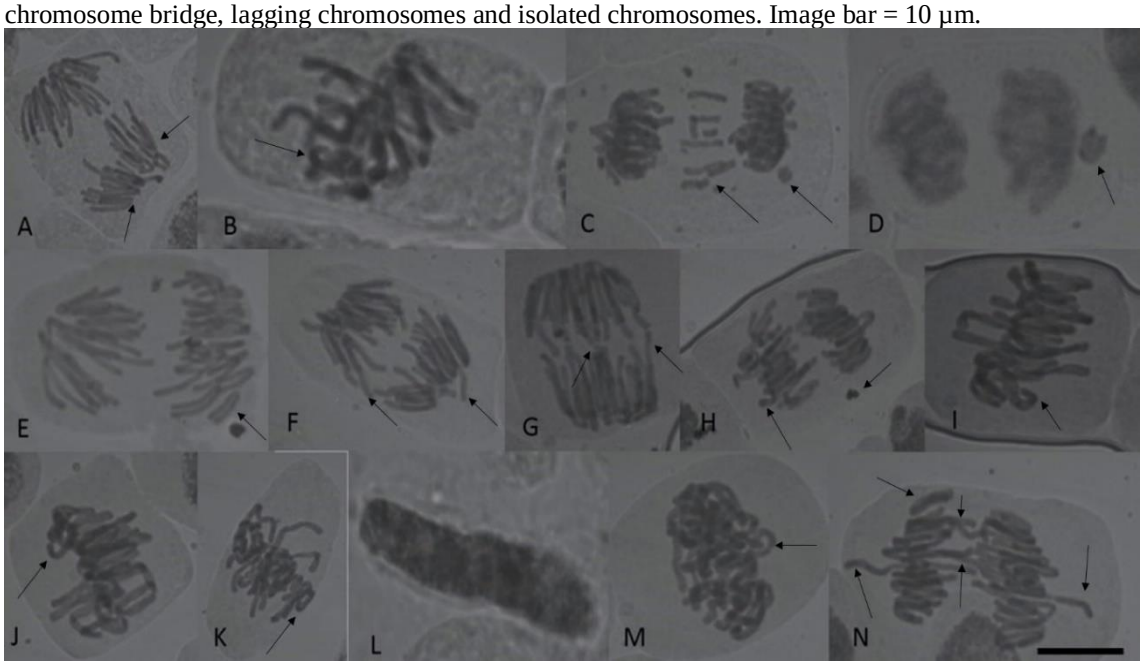

Table 6. Abnormalities found in infusions of *Costus scaber* via the *Allium cepa* biotest, using the direct and indirect method

	CHROMOSOMAL ABNORMALITIES						
	Micronucleus	Extended nucleus	Ring chromosome	Isolated chromosome	Retarded	C chromosome	Bridge
Direct Method	12	18	24	47	28	6	6
Indirect Method	18	39	39	69	71	0	14

Therefore, according to the literature and the results found in the present study, the species *C. scaber* has medicinal properties in secondary compounds that inhibit the proliferation of cancer cells. Since both the direct and indirect methods showed a high percentage antiprofilerative effect.

4 DISCUSSION

An increase in the mitotic index may be a consequence of a reduction in the time required for DNA repair (Evseeva et al., 2003). It can reveal uncontrolled proliferation of cells, which may end in tumor formation (Hoshina, 2002; Causil et al., 2017), i.e., indicative of a reduction in the duration of the mitotic cycle (Al-Ahmadi, 2013). Any one of these options can be characterized as being harmful to cells (Borah and Talukdar, 2002; Madaan and Mudgal, 2011). For the concentrations/exposure periods of *C. scaber* analyzed, no mitotic index results of the samples was observed as higher than that of the negative control. However, it was observed that the concentrations of *C. scaber* presented MI well below the negative control MI, which is an indication that the respective concentrations inhibit the normal cell division of cells, and may also inhibit the uncontrolled cell cycle of human cancer cells.

In the study by Taylor et al. (2006), in which they evaluated the stem extract of costus scaber, it was found that this extract was highly specific and potent (at 10 µg/ml) against the SKBR3 breast cancer cell line. *C. scaber* extract had no effect on the activity of proteolytic enzymes. However, in other *Costus* species, compounds with anti-inflammatory and antitumor activities, such as diosgenin in *C. speciosus*, have been reported. Anti-inflammatory, estrogenic and mastogenic activities have also been reported for species of this genus. The compounds found in this genus should be studied in order to safely use anti-inflammatory and anticancer drugs in traditional medicine. The study by Taylor et al. highlights the importance of medicinal studies of the species of the

genus *Costus*, since these are widely used in folk medicine and may also have broader use in pharmacological and oncological studies.

The determination of the mitotic index (MI) (or rate of cell division) in meristematic zones are very useful in order to determine the "state of health" and meristematic activity of cells (Fiskesjö, 1985). This is the main reason why the *Allium cepa* method has been widely used, especially when inhibition of root growth is observed (Dayan et al., 2000). As seen in Table 3, with regard to root size for all concentrations and exposure periods of the species *C. scaber*, all mean root sizes were smaller than the negative control. Since in the exposure period of 48 hours the inhibition of root growth was higher, this indicates that there was greater toxicity when compared to the exposure period of 72 hours. It is also known that concentrations in the exposure period of 48 hours showed greater toxicity than the positive control.

From the macroscopic analyses in the study by Sousa et al. (2017), it was possible to observe that the extracts of leaves and stems of *C.spiralis* showed inhibitory effect on the growth of roots of *A. cepa*, similar to what was observed for the positive control in our study. The meristems of the roots of onions treated with infusions from leaves or stalks of *C. spiralis* showed signs of toxicity, presenting changes in color, marks of necrosis, curving and tumors, corroborating with the present study. Since the species *C. scaber* belongs to the same genus as *C. spiralis*, it also showed inhibition in root growth for both exposure periods and in all the concentrations.

It is known that cancer is responsible for about 13% of deaths in the world, and, each year, more than 7 million people die from this disease (Souza et al., 2017). It is a class of disease where a cell faction exhibits uncontrolled growth, invasion, and sometimes metastasis. These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, do not invade or metastasize. Most cancers form a tumor, but some, such as leukemia, do not have such a formation (Souza and Böing, 2018).

The extracts of the leaves of some species of the genus *Costus* L. have antitumor activity that can contain the growth of many types of cancer including breast cancer. Raw *Costus* leaf extracts have *in vitro* inhibitory effects for various human cancers, including colon cancer, lung cancer, breast cancer, hepatoma and skin cancer (Dhanasekaran et al., 2014).

In addition, it was found that the raw extract of *Costus* leaves has antitumor activities *in vitro* and *in vivo*. Pharmacological studies have shown that the leaves of

Costus igneus have antibacterial, anticancer, and antidiabetic properties, as well as antioxidant properties (Dhanasekaran et al., 2014).

However, the compounds present in the leaves of some species of *Costus* can be harmful to the body when the plant is used indiscriminately, since chromosomal changes can occur in the meristematic cells of *Allium cepa* (bioindicator). These changes are considered as being the final result of the genotoxic effects of various physical and chemical agents and are also estimates of exposure levels of various organisms to these agents that harm human health (Pohren et al. 2013).

Some abnormalities caused in the analyzed treatments can be explained by Heald et al. (2018), who affirm that the TPX2 protein is one of the proteins responsible for the assembly of the mitotic spindle. When it is unable to perform assembly due to the genotoxic effect caused by infusions of *C. scaber*, it causes a decrease in the spindle. Then the Kif2a protein tries to repair the damage, if it is not possible to reverse the situation then the anomalies arise, and the cell blocks the division process mainly in the anaphase, causing irregularities such as lagging and chromosomal breaks (Macedo et al., 2018).

Chromosomal bridges and breaks were one of the main abnormalities observed in the root cells of *A. cepa*. According to Türkoglu (2007), adhesion is a common sign of the presence of toxic effects in genetic material and can cause irreversible effects in the cell. However, in this study, the presence of this abnormality may explain the significant induction of chromosomal bridges and breaks. Some of the bridges observed were probably formed from adhesion, since they were not accompanied by fragments (Kong and Ma, 1999). During the anaphase, these bridges can break down and form lagging chromosomes (Gömürgen, 2005).

Chromosomal abnormalities are caused due to the toxic effect on DNA (synthesis or replication) or nucleoproteins, which can lead to the effect of direct breaking of chromosomes or abnormal segregation of chromosomes. Anomalies can be widely classified as clastogenic changes (direct breaking effect on chromosomes) and physiological (effect on spindle proteins). Clastogenic effects are observed in the form of chromosomal/chromatid breaks, rings and bridges (Gömürgen, 2005; Gupta et al., 2019).

The mutagenic activity of infusions was also evaluated by the presence of micronuclei, which are the result of chromosomal breaks and disturbances in the mitotic process due to malfunctioning of the mitotic spindle (Grover and Kaur, 1999). Also micronucleated cells can arise from nuclear buds, which are eliminated from the nucleus

through an active process during the mitotic cycle of the cell in the S phase (Shimizu et al., 1998; Cox and White, 2019).

In the present study, ring chromosomes were also found, which is the result of broken ends of chromosomes that unite to form the ring chromosome (Peacock et al., 1973; Raghuvanshi and Singh, 1976; Mendes et al., 2019). The aforementioned authors also state that among the physiological anomalies, during the separation of chromosomes, one chromosome moves ahead of the others and separates from the rest of the group of chromosomes, and this anomaly is called the accelerated chromosome. While the lagging is a situation where in the separation, a chromosome lags behind the rest of the group as it does not attach to the axis fiber. The laggers and the accelerated ones result in unequal distribution of chromosomes in the daughter cells. Sometimes the separation of chromosomes does not occur during anaphase and all chromosomes lie near the equatorial plate, which is recorded as anaphase lagging.

When a genotoxic agent attacks a meristematic cell, it causes damage to the genetic functioning of the cell, this damage is repaired through the cellular mechanism of repair or, if not repaired, this leads to various types of chromosomal abnormalities (as observed in Figure 1 and Table 6).

5 CONCLUSION

It can be confirmed that the species *C. scaber* presents cytotoxic and genotoxic effects, in addition to having an antiprofilerative effect of a high percentage in some of the concentrations used. It can be used in the genetic improvement of plants for use in pharmacology, especially in the field of oncology, since it has secondary compounds that inhibit cell division.

In addition, for the population that uses this species as a herbal remedy, it is recommended that strong concentrations are not used or that this remedy is used during several consecutive days, since it can cause harm to the human organism due to a high cytotoxic and genotoxic index in some concentrations as the exposure period increased.

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