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# *In vivo* assessment of cyto/genotoxic, antigenotoxic and antifungal potential of *Costus spiralis* (Jacq.) Roscoe leaves and stems

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**ABSTRACT**

*Costus spiralis* is a Brazilian native plant used in popular medicine, but the safety of this therapeutic use needs investigation. So, the aim of this study was to evaluate the cytogenotoxic and antigenotoxic effects of *C. spiralis* leaves or stems aqueous extracts on *Allium cepa* root cells. Moreover, a phytochemical screening and an antioxidant and antifungal activities evaluation were performed. *C. spiralis* aqueous extracts presented cytotoxicity, but no mutagenicity was observed. When the antigenotoxicity was evaluated, *C. spiralis* leaves aqueous extract presented preventive and modulatory effects on *A. cepa* root cells, reducing the sodium azide cytogenotoxic effects. In contrast, *C. spiralis* stems aqueous extract enhanced the sodium azide cytogenotoxicity in some conditions. The phytochemical screening revealed the presence of phenolic compounds in *C. spiralis*. When total phenolic content was determined, the leaves presented 73% more phenolic content than stems. Corroborating this data, *C. spiralis* leaves antioxidant potential was 30% higher than *C. spiralis* stems. However, these extracts did not present antifungal activity against *Candida* spp. In conclusion, empirical utilization of *C. spiralis* aqueous extracts should be avoided. Moreover, the cytotoxic effect of *C. spiralis* leaves and stems can play an important role in anticancer therapy and must be deeply studied.

**Key words:** *Allium cepa* test, antioxidant, medicinal plant, secondary metabolites.

## INTRODUCTION

*Costus spiralis* (Jacq.) Roscoe, popular known as cana-de-macaco or cana-do-brejo, is a Brazilian native plant found in Cerrado, Amazon, Atlantic Forest and Caatinga biomes (Maas and Maas 2015). This species is a member of Costaceae family which is commonly used in popular medicine to treat urinary infections and kidney stones (Pilla et al. 2006). The leaves infusion is used to treat

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arterial hypertension and as diuretic and the leaves decoction is used against diarrhea. Stems infusion is used to treat hepatitis and abdominal pain. Topically, the plant is used to treat syphilis wounds and tumors (Di Stasi and Hiruma-Lima 2002).

Besides the use of *C. spiralis* by the population, there is also some information about its therapeutic potential on the literature. For example, the antiurolithiatic activity of *C. spiralis* aqueous extract significantly reduced the growth of calcium oxalate calculi in the urinary bladder of rats (Viel et al. 1999), which is in agreement with its traditional use. It was also demonstrated that the *C. spiralis* leaves aqueous fraction reduces mammalian myocardium contractility by impairing the calcium inward current in this muscle. This data support the use of *C. spiralis* in arterial hypertension or cardiac arrhythmias treatment (Britto et al. 2011). The antioxidant activity of dichloromethane and methanol extracts of *C. spiralis* rhizome and root was also demonstrated. Only the dichloromethane extract presented antibacterial activity in this work (Habsah et al. 2000). In accordance, *C. spiralis* leaves aqueous extract presented antimicrobial activity against different *Vibrio cholerae* strains (Pérez et al. 2008). Steroidal saponins isolated from *C. spiralis* fresh rhizomes inhibited the increase in vascular permeability caused by acetic acid, a typical model of first stage inflammatory reaction, suggesting an anti-inflammatory activity (Silva and Parente 2004).

All those therapeutic activities observed in *C. spiralis* extracts can be intrinsically related to the secondary metabolites produced by this plant. It is known that the methanolic extract of *C. spiralis* leaves presents flavonoids, sterols and alkaloids (Braga et al. 2007). The aqueous fraction instead presents phenols, tannins, flavones, xanthones, flavonoids, flavonols, flavononols, flavonones, and saponins (Britto et al. 2011). Saponins and flavonol glycosides were isolated from *C. spiralis* rhizome and leaves, respectively (Antunes et al. 2000, Silva and Parente 2004). All those compounds are able to increase the plant survival, since they can present antimicrobial, antiviral and antigerminative activities (Gobbo-Neto and Lopes 2007). The secondary metabolites can also be used to improve human health, preventing mutagenic, cytotoxic and genotoxic effects (Sun et al. 2015). On the other hand, these substances could induce genotoxic alterations, what could cause different pathologies, such as cancer (Sakihama et al. 2002).

Most plants with therapeutic activities were not sufficiently studied regarding to cytogenotoxic/mutagenic actions, and these parameters are important to ensure the safety of therapeutic use (Tedesco et al. 2015). The *Allium cepa* L. test system is widely used for evaluating the cytogenotoxic potential of infusions made with medicinal plants (Bagatini et al. 2007). Some studies point to a high concordance between this vegetal and mammal systems (Bagatini et al., 2007, Sturbelle et al. 2010, Fedel-Miyasato et al. 2014, Roberto et al. 2016). Besides that, it is a low cost and easy-handling assay (Leme and Marin-Morales 2009), which allow to expose the test organism directly to complex mixtures without previous treatment of the test sample (Rank and Nielsen 1993). The present study aimed to evaluate the cytogenotoxic and antigenotoxic effects of crude aqueous extracts of *C. spiralis* leaves or stems on the cell cycle of *A. cepa* roots at different concentrations and exposure protocols. Moreover, the phytochemical screening and antioxidant and antifungal activities evaluation of these extracts were performed.

## MATERIALS AND METHODS

### PLANT MATERIAL

*C. spiralis* leaves were collected from Israelândia in Goiás State (16º19’21.3”S 50º54’20.0”W, 383 m), Brazil, in August 2013. The plant was identified by Dr. Aristônio Magalhães Teles, a taxonomist from Universidade Federal de Goiás, Brazil. A voucher

specimen was deposited at the Herbarium of the Universidade Estadual de Goiás (9208). *C. spiralis* leaves and stems were dried at room temperature and powdered in a knife mill. The powdered material was stored from light and moisture was used for subsequent extraction process. For this, the leaves or stems were first soaked during 10 min in sterile water and then boiled during 5 min. Three different concentrations of *C. spiralis* leaves (18 mg/ml, *Cs*L18; 9 mg/ml, *Cs*L9; and 4.5 mg/ml, *Cs*L4.5) or stems (18 mg/ml, *Cs*S18; 9 mg/ml, *Cs*S9; and 4.5 mg/ml, *Cs*S4.5) aqueous extracts were used in this work. These concentrations were chosen based on *Costus spicatus* popular use (Keller et al. 2009), since no scientific references for *C. spiralis* was found and based on a taxonomic proximity between these species.

*Allium cepa* TEST

The *Allium cepa* test was adapted from the method reported by (Fiskesjo 1985). Briefly, *A. cepa* bulbs were exposed to three different concentrations of *C. spiralis* leaves (*Cs*L18, *Cs*L9 and *Cs*L4.5) or stems (*Cs*S18, *Cs*S9 and *Cs*S4.5) aqueous extracts. Sterile mineral water and paracetamol 0.8 mg/ml or sodium azide 2 g/l were used as negative (NC) and positive (PC) controls, respectively. After 24 h of exposure, roots were collected for microscopic evaluation. For this, 1000 cells were evaluated from each bulb, totalizing 6 bulbs per treatment (6000 cells). After 72 h, the roots were collected for macroscopic evaluation to assess toxicity. For this, the roots were measured with a pachymeter and the following parameters were observed: changes in tissue color, bending or twisting of the roots and presence of tumors.

*Cytogenotoxic evaluation*

For cytogenotoxic evaluation, the roots were collected, washed with sterile mineral water and placed in Carnoy's fixative solution (3:1 ethanol:glacial acetic acid v/v) refrigerated at 4 °C for 24 h, followed by 70% ethanol solution and refrigeration. The roots were subsequently hydrolysed in HCl 1N for 10 min and washed with sterile mineral water. After this, the roots were placed on a microscope slide and one drop of acetocarmine 1% was added. Then, the roots were macerated with rusty needles (Mondin and Neto 2006). A coverslip were placed on the material and a pressure was made to help separate the cells. The slides were evaluated using a Zeiss Primo Star® optical microscope with magnification of 40 or 100x. The following parameters were observed: (a) mitotic index (MI) and the frequency of (b) chromosomal aberrations (CA), (c) micronuclei (MN) and (d) nuclear abnormalities (NA). MI was calculated as follow:

$$\mathrm{MI}(\%) = \frac{number\ of\ cells\ in\ mitosis}{1000} x100$$

*Antigenotoxic evaluation*

The *A. cepa* test was also used to evaluate the antigenotoxicity of the *C. spiralis* aqueous extract. Here sodium azide 2 g/l 24 h exposition was used as positive control (PC) because of its bigger mutagenic potential, and sterile mineral water 24 h exposition as negative control (NC). Three types of exposure protocols were performed: (1) pre-treatment, in which the *A. cepa* bulbs were exposed initially to *C. spiralis* leaves or stems aqueous extract for 24 h and the roots were then washed in distilled water and placed in sodium azide solution for 24 h; (2) co-treatment, in which the bulbs were exposed to *C. spiralis* leaves or stems aqueous extracts and sodium azide solution for 24 h; and (3) post-treatment, in which the bulbs were first placed in sodium azide solution for 24 h, and then the roots were rinsed with distilled water and exposed to *C. spiralis* leaves or stems aqueous extracts for 24 h. The samples were then processed in the similar way as mentioned above.

### STATISTICAL ANALYZES

Data belonging to the same group (leaves or stems and pre-, co- or post-treatment) were compared with negative and positive controls using one-way ANOVA and Tukey's pairwise comparison. For this, Microsoft® Excel® for Mac 2011 was used to calculate average and standard deviation of the data and PAST version 1.94 (Hammer et al. 2001) was used to compare the means. We considered statistically significant p values < 0.05.

### PHYTOCHEMICAL SCREENING

We evaluated the presence of the following secondary metabolites: alkaloids (Dragendorff Hager and Mayer reaction), anthraquinones (acid/base reaction), coumarins (under UV light observation), flavonoids (cianidin and sulfuric acid reactios, A-1 and A-II), phenolic compounds (precipitation reaction with ferric chloride), tannins (iron salts reaction, protein precipitation, B-1 and B-II), and saponins (Lieberman-Buchard reaction and foam index), using methodologies previously described (Matos 1988, Matos and Matos 1989, Costa 2001).

### DETERMINATION OF TOTAL PHENOLS

For total phenols determination, aqueous extracts were prepared from 0.75 g of the sample (leaves or stems). This material was transferred to a 250 ml erlenmeyer flask and 150 ml of distilled water were added. The mixture was heated to boiling and kept in a water bath at between 80 and 90 ºC for 30 min. After cooling, the contents of the flask were transferred to a 250 ml volumetric flask and the volume was made up with distilled water. The flask was then left for sedimentation and the liquid was filtered through qualitative filter paper. The first 50 mL of filtrate was discarded. Each sample was prepared in triplicate. The aqueous extracts obtained were used for the determination of total phenols by the method of Hagerman & Butler adapted by Waterman and Mole (1994).

### ANTIOXIDANT ACTIVITY (AOA)

The scavenging activity of the DPPH free radical was performed according to the adapted method described by Sánchez-Moreno and colleagues (1998). The samples were diluted in the same solution resulting in the concentrations 9 to 1.8 mg/ml. After, 0.1 ml of each solution was mixed with 3.9 ml of a 60 µM DPPH solution. After an incubation time of 30 min at room temperature, the absorbances were measured at 515 nm (Asample). The blank was performed with methanol without DPPH (Ablank). A control solution was performed using 3.9 ml DPPH solution and 0.1 ml of methanol (Acontrol). The scavenging activity of each solution was determined according to the following equation:

$$AOA(\%) = 100 - \frac{(Asample - Ablank)x100}{Acontrol}$$

AOA was finally expressed as $IC_{50}$, which means the concentration (mg/ml) of the extract required to cause a 50% decrease in initial content of the DPPH solution. The assays were performed in triplicate.

### AGAR-WELL DIFFUSION ASSAY

Two *Candida* species were tested in this work: *Candida albicans* (ATCC 28367) and *Candida parapsilosis* (ATCC 22019). The yeast cells were cultivated on Sabouraud dextrose agar medium (Himedia Laboratories Pvt. Ltd., India) at room temperature. Every 48 h, the yeast cells were put in a fresh medium for maintenance. Fresh cells were used to produce an inoculum in sterile saline solution (NaCl 0.9%). The cell suspension was standardized in spectrophotometer (BEL Photonics 2000 UV) at 625 nm to 0.5 in McFarland scale. The antifungal activity was performed using the agar-well diffusion assay in triplicate. Briefly, Sabouraud

dextrose agar plates with 10 µg/ml gentamicin were seeded with the cell suspension prepared. After this, 5 wells 6 mm in diameter each were made with help of a sterile tip. Each well was filled with 50 µl of *C. spiralis* leaves (*Cs*L18, *Cs*L9 and *Cs*L4.5) or stems aqueous extracts (*Cs*S18, *Cs*S9 and *Cs*S4.5). Fluconazole (Halex Istar Indústria Farmacêutica LTDA., Brasil) 2 mg/ml and sterile water were used as positive and negative controls, respectively. The plates were incubated at room temperature for 48 h. Inhibition halos were measured using a pachymeter and compared with the positive and negative controls.

## RESULTS

### CYTOGENOTOXIC EVALUATION

*C. spiralis* leaves or stems aqueous extracts presented inhibitory effect on *A. cepa* root's growth. This inhibition seems to be concentration-dependent, since the smallest concentration from leaves or stems inhibited *A. cepa* root's growth less than the highest concentration **(Fig. 1a)**. The roots

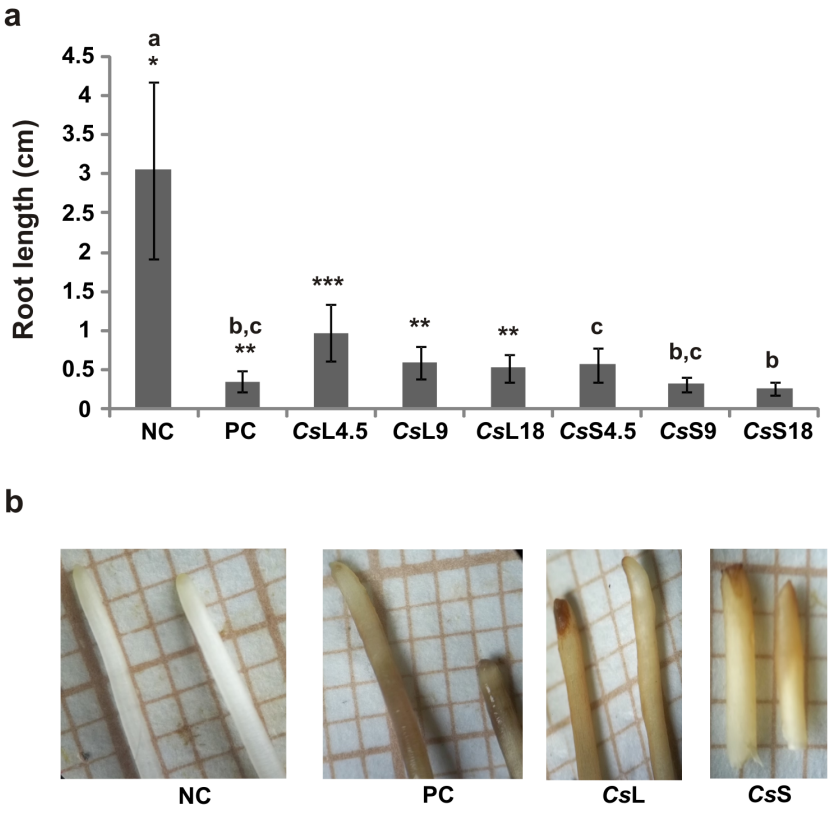


**Figure 1 - *Costus spiralis* aqueous extracts toxicity against *Allium cepa* meristematic root cells.** *A. cepa* bulbs were exposed to *C. spiralis* leaves or stems aqueous extracts for 72 h. After this time, the root's growth was evaluated. The mean and standard deviation of *A. cepa* root length after *C. spiralis* extracts exposure are presented (**a**). A representative image of treatments is also exhibited, showing changes in color, necrosis marks, bending and tumors (**b**). NC: negative control; PC: positive control; *Cs*L4.5: 4.5 mg/ml leaves aqueous extracts; *Cs*L9: 9 mg/ml leaves aqueous extracts; *Cs*L18: 18 mg/ml leaves aqueous extracts; *Cs*S4.5: 4.5 mg/ml stems aqueous extracts; *Cs*S9: 9 mg/ml stems aqueous extracts; and *Cs*S18: 18 mg/ml stems aqueous extracts. Bars with different number of asterisks or letters represent statistically significant differences ($p < 0.05$) between leaves or stems treatments, respectively, and controls.

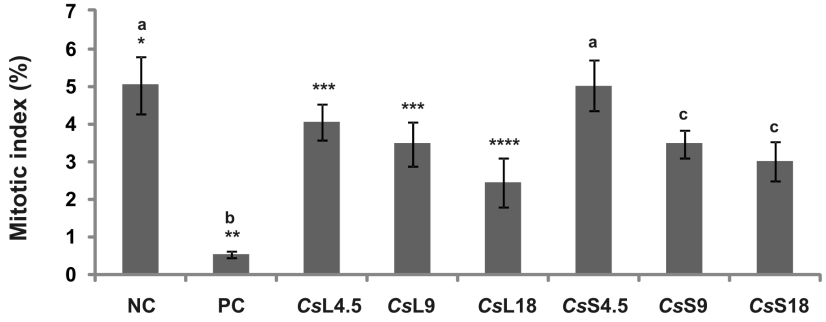


**Figure 2 - Cytotoxic effects of *Costus spiralis* aqueous extracts against *Allium cepa* meristematic root cells.** After 24 h exposure to *C. spiralis* leaves or stems aqueous extracts, *A. cepa* root cells were microscopically evaluated, considering interphase cells x cells in mitotic division. The graphic represents the mean percentage and standard deviation of the mitotic index. NC: negative control; PC: positive control; *Cs*L4.5: 4.5 mg/ml leaves aqueous extracts; *Cs*L9: 9 mg/ml leaves aqueous extracts; *Cs*L18: 18 mg/ml leaves aqueous extracts; *Cs*S4.5: 4.5 mg/ml stems aqueous extracts; *Cs*S9: 9 mg/ml stems aqueous extracts; and *Cs*S18: 18 mg/ml stems aqueous extracts. Bars with different number of asterisks or letters represent statistically significant differences ($p < 0.05$) between leaves or stems treatments, respectively, and controls.

presented changes in color, necrosis marks, bending and tumors when treated with these extracts **(Fig. 1b)**, indicating toxicity of *C. spiralis* extracts.

Corroborating the macroscopic data, the microscopic analysis revealed a statistically significant reduction on the MI of the organisms treated with *C. spiralis* leaves or stems aqueous extracts when compared to the negative control **(Fig. 2)**. It indicates a cytotoxic effect of the concentrations of *C. spiralis* extracts used in this work, with exception of *Cs*S4.5. In regard to *C. spiralis* leaves aqueous extract, the MI inhibition seems to be concentration-dependent **(Fig. 2)**. On the other hand, *C. spiralis* extracts did not induce CA or MN mutagenicity in *A. cepa* root meristems. Only 0.081% of analyzed cells presented a genotoxic alteration, indicating no genotoxic or mutagenic activity of the tested extracts.

## ANTIGENOTOXIC EVALUATION

When *A. cepa* root meristems were initially exposed to *C. spiralis* leaves or stems aqueous extracts in pretreatment and were posteriorly treated with sodium azide solution, *C. spiralis* leaves aqueous extract was able to prevent the cytotoxicity and mutagenicity of the sodium azide **(Fig. 3a and Fig. 4a)**. The cytotoxic prevention of *C. spiralis* leaves aqueous extract seems to be concentration-dependent. However, *C. spiralis* stems aqueous extract not only failed to prevent the sodium azide cytotoxicity, but also enhanced this cytotoxicity in a concentration-dependent manner **(Fig. 3b)**. Regarding MN frequency, the highest concentration of *C. spiralis* stems aqueous extract (*Cs*S18) also enhanced the sodium azide mutagenicity. In contrast, the other concentrations of *C. spiralis* stems aqueous extract used in this work (*Cs*S4.5 and *Cs*S9) prevented the sodium azide mutagenicity **(Fig. 4b)**.

In co-treatment, *C. spiralis* leaves aqueous extract modulated the cytotoxicity and mutagenicity of sodium azide in a concentration-independent manner, increasing the root meristem cell multiplication and decreasing the MN frequency, respectively, when compared to the positive control **(Fig. 3a and Fig. 4a)**. In regard to *C. spiralis* stems aqueous extract, we could not observe a significant modulation of sodium azide cytotoxicity and mutagenicity, with exception of the highest

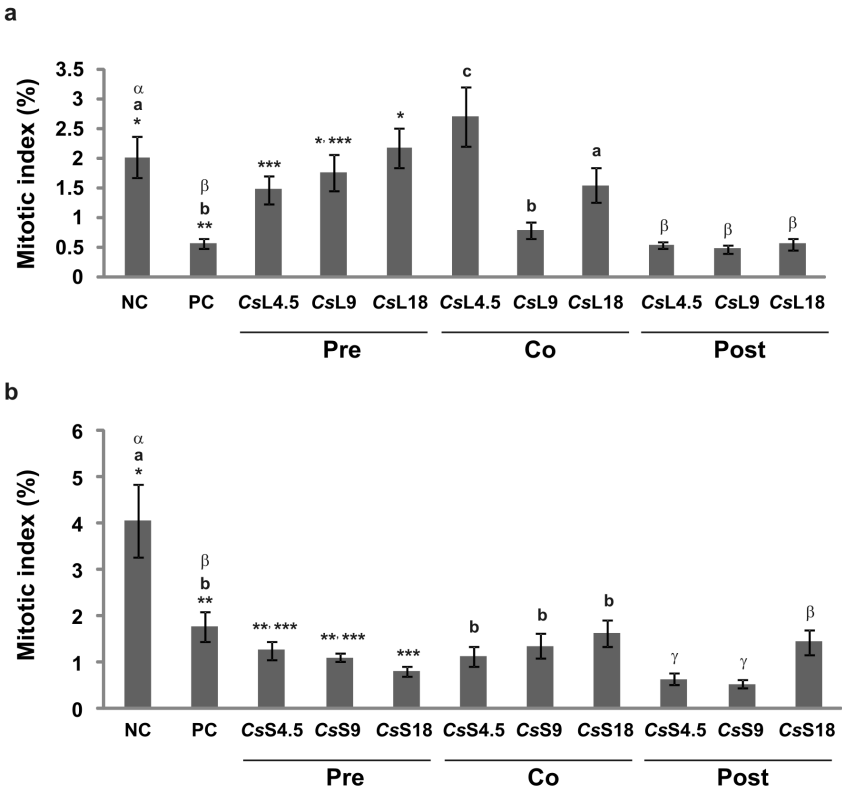


**Figure 3 - Anticytotoxic effects of *Costus spiralis* aqueous extracts against *Allium cepa* meristematic root cells.** The anticytotoxic evaluation was performed using sodium azide as positive control in three different exposure protocols: pre-treatment, co-treatment and post-treatment with *C. spiralis* leaves (**a**) or stems (**b**) aqueous extracts. The data is represented as mean percentage and standard deviation of the mitotic index. NC: negative control; PC: positive control; *Cs*L4.5: 4.5 mg/ml leaves aqueous extracts; *Cs*L9: 9 mg/ml leaves aqueous extracts; *Cs*L18: 18 mg/ml leaves aqueous extracts; *Cs*S4.5: 4.5 mg/ml stems aqueous extracts; *Cs*S9: 9 mg/ml stems aqueous extracts; and *Cs*S18: 18 mg/ml stems aqueous extracts. Bars with different number of asterisks or roman letters or greek letters represent statistically significant differences ($p < 0.05$) between leaves (**a**) or stems (**b**) treatments and controls in pre-, co- or post-treatment, respectively.

concentration (*Cs*S18) that enhanced the sodium azide mutagenicity **(Fig. 3b and Fig. 4b)**.

In post-treatment, *C. spiralis* aqueous extracts failed to inhibit the cytotoxic activity of sodium azide **(Fig. 3)**. Contrariwise, the lower concentrations of *C. spiralis* stems aqueous extract (*Cs*S4.5 and *Cs*S9) also enhanced the sodium azide cytotoxicity **(Fig. 3b)**. Regarding MN frequency, *C. spiralis* leaves aqueous extracts enhanced the mutagenicity potential of sodium azide **(Fig. 4a)**. While just the lower concentration of *C. spiralis* stems aqueous extract (*Cs*S4.5) enhanced the sodium azide mutagenicity **(Fig. 4b)**.

Some other cellular alterations, like necrosis, CA and NA were also observed **(Fig. 5)**, but their frequency was not relevant in this work.

## PHYTOCHEMICAL SCREENING AND PHENOL QUANTIFICATION

The phytochemical screening of *C. spiralis* leaves and stems revealed the presence of phenolic compounds, flavonols, flavanones, flavones,

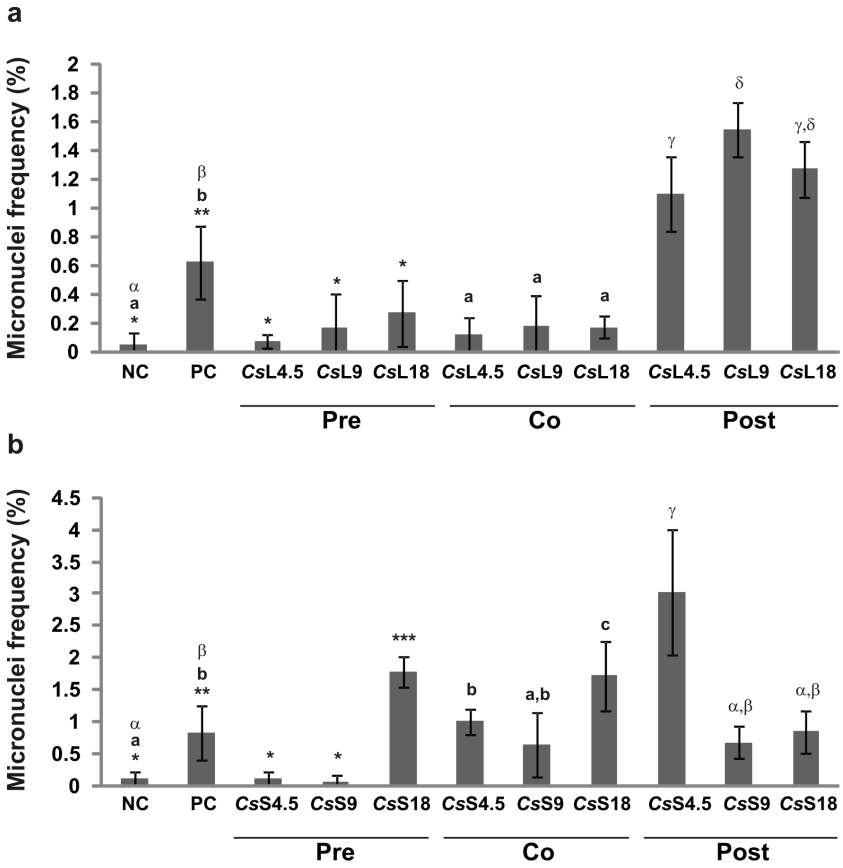


**Figure 4 - Micronuclei frequency on *Allium cepa* meristematic root cells exposed to *Costus spiralis* leaves or stems aqueous extracts.** This evaluation was performed using sodium azide as positive control in three different conditions: pre-treatment, co-treatment and post-treatment with *C. spiralis* leaves (**a**) or stems (**b**) aqueous extracts. The data is represented as mean percentage and standard deviation of micronucleus frequency. NC: negative control; PC: positive control; *Cs*L4.5: 4.5 mg/ml leaves aqueous extracts; *Cs*L9: 9 mg/ml leaves aqueous extracts; *Cs*L18: 18 mg/ml leaves aqueous extracts; *Cs*S4.5: 4.5 mg/ml stems aqueous extracts; *Cs*S9: 9 mg/ml stems aqueous extracts; and *Cs*S18: 18 mg/ml stems aqueous extracts. Bars with different number of asterisks or roman letters or greek letters represent statistically significant differences ($p < 0.05$) between leaves (**a**) or stems (**b**) treatments and controls in pre-, co- or post-treatment, respectively.

tannins and saponins **(Table I)**. The total phenolic content was determined, indicating 1.23% and 0.71% in *C. spiralis* leaves and stems, respectively.

ANTIOXIDANT EVALUATION

Considering the high amount of phenolic compounds present in *C. spiralis* leaves and stems, we decided to investigate the antioxidant potential of this plant. Using DPPH radical scavenging method, we observed a $IC_{50}$ = 11.82 mg/ml to leaves and $IC_{50}$ = 15.38 mg/ml to stems, comparing with tannic acid $IC_{50}$ of 0.88 mg/ml. This result suggests that the antioxidant potential of *C. spiralis* leaves is 30% higher than *C. spiralis* stems.

ANTIFUNGAL EVALUATION

Observing the *C. spiralis* cytotoxic potential, we decide to evaluate the antifungal activity of *C.*

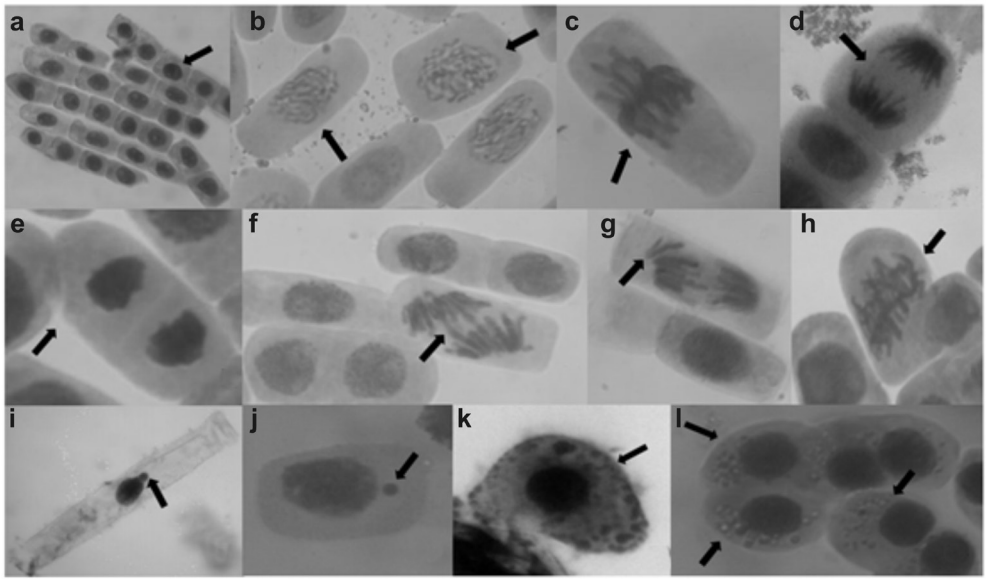


**Figure 5 - Meristematic root cells in different mitosis phases and abnormalities probably induced by *Costus spiralis* leaves or stems exposure.** Normal cells in interphase (**a**), prophase (**b**, arrow), metaphase (**c**, arrow), anaphase (**d**, arrow) and telophase (**e**, arrow). Chromosomal aberrations, like chromosome bridges (**f**, arrow), chromosome losses (**g**, arrow) and chromosome adherence (**h**, arrow) were observed. Nuclear abnormalities, like nuclear bud (**i**, arrow), micronuclei (**j**, arrow) and cellular alterations, like apoptotic cells (**k**, arrow) and cells in necrosis (**l**, arrow) were also observed in this work.

**TABLE I**
**Investigation of secondary metabolites classes in *Costus spiralis* leaves or stems.**

| Secondary metabolites | Leaves | Stems |
|---|---|---|
| Phenolic compounds | + | + |
| Flavonols, Flavanones | + | + |
| Flavones (cyanidin) | + | + |
| Tannins | + | + |
| Coumarins | - | - |
| Alkaloids | - | - |
| Anthraquinones | - | - |
| Saponins | + | + |

+ presence.
- absence.

*spiralis* leaves or stems aqueous extracts. The concentrations of *C. spiralis* extracts used in this work showed no inhibitory activity against *C. albicans* or *C. parapsilosis*, when compared with the fluconazole positive control. The fluconazole inhibition zone diameter was 1.6 cm $\pm$ 0.15 and 1.4 cm $\pm$ 0.23 against *C. albicans* and *C. parapsilosis*, respectively **(Fig. 6)**.

## DISCUSSION

The use of herbal medicinal products and supplements has increased over the past three decades. In developed countries, herbal therapy is used with the expectation that it will promote healthier living. In developing countries, herbal medicine is an integral part of the culture of communities because synthetic drugs are imported, have high costs, and thus are inaccessible to majority of the population (Bailão et al. 2015). However, most of them were not deeply studied regarding the safe use by population. Some species could present cytotoxic or mutagenic substances in their composition that could be harmful to humans (Bagatini et al. 2007). In this study, the cytogenotoxic potential of *C. spiralis* leaves or stems aqueous extracts was investigated. Using *A. cepa* bioassay, both *C. spiralis* leaves and stems aqueous extracts seems to present concentration-dependent cytotoxic effects. However no genotoxic

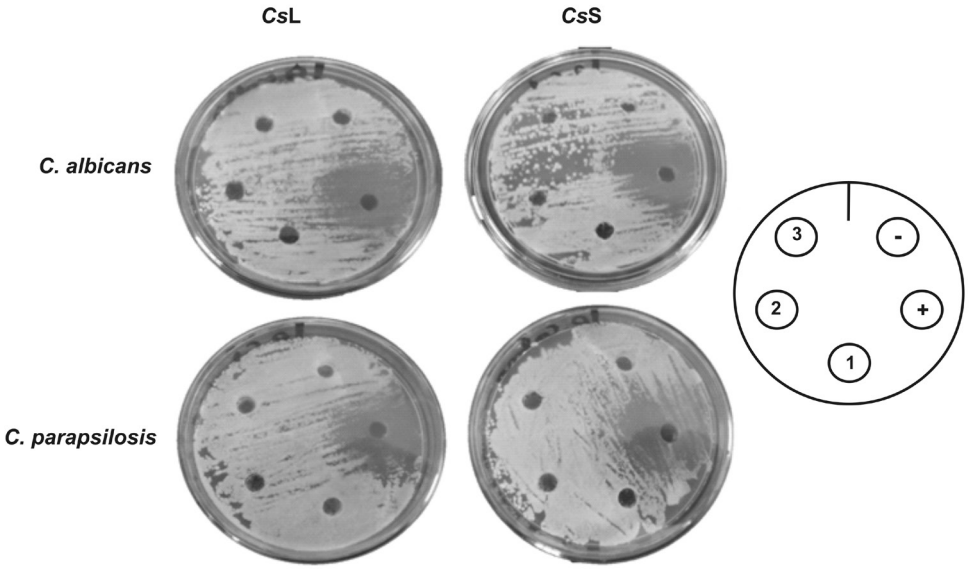


**Figure 6 -** ***Costus spiralis*** **leaves or stems aqueous extract presented no antifungal activity against** ***Candida*** **spp.** *C. albicans* and *C. parapsilosis* were cultivated on Sabouraud Dextrose agar for 48 h. Then, the yeasts were spreaded on this media in presence of three different concentrations of *C. spiralis* leaves (***Cs*****L**) or stems (***Cs*****S**) aqueous extract: 18 mg/ml (**1**), 9 mg/ml (**2**) or 4.5 mg/ml (**3**). After 48 h, the zones of inhibition were measured using a ruler. Fluconazol and distilled water were used as positive (+) and negative (-) control, respectively.

effect was observed in the concentrations tested in this work, based on this *in vivo* vegetal test system. The reduction of mitotic activity of *A. cepa* meristematic cells in presence of *C. spiralis* aqueous extracts may be due to inhibition of DNA synthesis or a blocking of cells in G2 phase as a result of damage in DNA (Sudhakar et al. 2001). Maybe because of G2 phase arrest, we could not observe a high amount of CA and NA in presence of *C. spiralis* aqueous extracts. Delay in DNA damage checkpoints provides time for the damaged DNA to be repaired, after which the cell-cycle brakes are released and progress resumes (Hartwell and Weinert 1989).

Similarly to what was observed in this work, *Solidago microglossa* DC. (Asteraceae) leaves infusion presented cytotoxic but not mutagenic effect using *A. cepa* test. *S. microglossa* is a vegetal species which contains tannins and flavonoids, among other substances, in its chemical composition (Bagatini et al. 2009). Tannins act in protein precipitation and enzymatic inhibition. So, the enzymatic interference assigned to tannins may be responsible for the inhibition of *A. cepa* meristematic cell division observed both in this work and in *S. microglossa* study (Teixeira et al. 2003, Bagatini et al. 2009). Some phenolic compounds, such as flavonoids, act as prooxidant mainly in systems that contain redox-active metals, which in presence of $O_2$ may led to ROS and phenoxyl radicals formation, which damage DNA and other biomolecules (Shi et al. 2001, Galati and O'Brien 2004). So, the presence of these secondary metabolites could also contribute to *C. spiralis* cytotoxic activity against *A. cepa* meristematic cells. Corroborating this hypothesis, a phytochemical screening performed in this work revealed the presence of phenolic compounds, flavonols, flavanones, flavones, tannins and saponins in both *C. spiralis* leaves and stems.

When the antigenotoxicity was evaluated, we observed that the *C. spiralis* leaves aqueous extract presents a preventive and a modulatory effect on *A. cepa* root cells in presence of sodium azide and this extract was able to reduce the sodium azide mutagenic effect. However, this extract was not able to activate the repair system to correct mistakes on cell cycle promoted by the sodium azide and then enhanced sodium azide mutagenicity on post-treatment. Regarding the *C. spiralis* stems aqueous extract, we observed that this extract enhanced the sodium azide cytotoxicity and mutagenicity in some conditions.

These results suggest a dual-role to *C. spiralis* leaves aqueous extract, reducing sodium azide damage effects in some situations (pre- and co-treatment) or enhancing the sodium azide mutagenicity in others (post-treatment). Sodium azide is an inhibitor of catalase, peroxidase and cytochrome oxidase, thus influencing respiratory processes, increasing the oxygen free radicals and reducing the efficiency of DNA repair (Gruszka et al. 2012). Since sodium azide reduce the DNA repair efficiency, some phenolic compounds present in the *C. spiralis* leaves aqueous extract could be acting, during post-treatment, as a pro-oxidant or binding directly to DNA, promoting unrepaired clastogenic events what could induce micronuclei formation (Ferguson 2001).

The same phenolic compounds that act as a pro-oxidant, could act as an antioxidant agent (Cao et al. 1997), scavenging free radicals produced by sodium azide and then reducing the sodium azide mutagenic effect as observed in *C. spiralis* leaves aqueous extract pre- and co-treatment. Tannins, specially, are characterized by a reductive chemical structure that possesses the capacity for free radical sequestration (Silva et al. 2003). Ellagitannins and their hydrolyzed products, for example, present antimutagenic activity against sodium azide (Zahin et al. 2014, Carneiro et al. 2017), maybe because their antioxidant properties. Some alkaloids also present antioxidant activity (Ahmad et al. 2017). Thus, these metabolites could contribute to increase the antioxidant properties of *C. spiralis* extracts.

We also investigated if the difference observed between *C. spiralis* leaves or stems aqueous extracts regarding to antigenotoxic capacity was related to antioxidant activity. We observed that *C. spiralis* leaves present more total phenols and a higher $IC_{50}$ in DPPH scavenging method than *C. spiralis* stems. A strong correlation between total phenolic content and antioxidant activity index has already been observed, with a correlation coefficient ($r^2$) of approximately 0.8 (Habermann et al. 2016). Moreover, leaves commonly present more total phenols and antioxidant activity index than stems (Basma et al. 2011, Mahdi-Pour et al. 2012, Habermann et al. 2016, Adebiyi et al. 2017). This way, phenolic compounds presented more in leaves than in stems of *C. spiralis* could be acting scavenging free radicals produced by sodium azide, suggesting that *C. spiralis* leaves aqueous extract possess more cell protector potential against sodium azide than the stem extract.

Despite *C. spiralis* leaves or stems aqueous extracts presented cytotoxic activity against *A. cepa* meristematic root cells, this extracts did not present antifungal activity against *Candida* spp. Similar results were previously observed. *C. spiralis* dichloromethane and methanol extracts were inactive against *C. albicans*, *Cryptococcus neoformans* and *Aspergillus ochraceous* (Habsah et al. 2000, Braga et al. 2007).

Since *C. spiralis* leaves or stems aqueous extracts presented cytotoxicity against *A. cepa* meristematic cells and could enhance the sodium azide cytogenotoxicity, it is important to investigate if these extracts present the same activities against mammal cells. Empirical utilization of *C. spiralis* aqueous extracts and co-administration of herb and therapeutic drugs in popular medicine could be harmful to human health and should be avoided. However, since *C. spiralis* demonstrated to be a

phenolic-rich raw material, it would be important to standardize phenolic extraction and separation methods thinking on pharmaceutical applications. The main bioactivities attributed to phenolic compounds are antioxidant, anti-microbial, anti-mutagenic, anti-angiogenic and anti-inflammatory (Sun et al. 2015). Moreover, cytotoxic effect of phenolic compound plays an important role in anticancer and antimitotic properties (Hadi et al. 2000). These possible applications turn *C. spiralis* and its metabolites an important target for further studies.

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