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Antitumoral activity, antioxidant capacity and bioactive compounds of ginger (Zingiber officinale)

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Abstract:

Zingiber officinale, popularly known as ginger, it is in National Relations of Medicinal Plants of Interest to SUS (RENISUS) aiming at the appropriate and safe use of medicinal plants. Then, it is necessary to characterize and determine their bioactive compounds, their cytotoxic, mutagenic and/or antitumor properties, in order to guarantee food safety and its beneficial coadjuvant effects. The ginger rhizomes were dried at temperatures 40, 60, and 80°C and the bioactive compounds were extracted with methanol and ethanol (70:30 and 95:5). The rhizomes showed to be intermediate source of vitamin C. The highest levels of anthocyanins and yellow flavonoids were dried at 40°C. The highest content of phenolic compounds was for the extracts MeOH 70:30 and EtOH 70:30 at 80°C and for flavonoids MeOH 95:5 at 40°C. Data showed a positive correlation with the content of bioactive compounds and the antioxidant activity. The EtOH 70:30 extract at 80°C, did not present a cytotoxic effect, by calculating the mitotic index, or mutagen, by the evaluation of the chromosomal alterations, for the *Allium cepa* L. This extract showed a cytotoxic effect for human kidney tumor cells, by MTT test, for all concentrations tested and in the two evaluation times. The data serve as basis for the consumption of ginger, with a high content of bioactive compounds, with no harmful effect for the normal cells studied and with antitumor potential.

Keywords: cytotoxicity, extraction, flavonoids, mutagenicity, phenolic compounds, tumor cells.

Introduction

For a long time, natural products have been used for the treatment, healing and prevention of diseases. Nowadays, about 25% of prescribed drugs are made from plants, and most pharmaceuticals are of natural origin, or synthesized from natural products (Barreiro & Bolzani, 2009; Oliveira, Oliveira, & Andrade, 2010; Andrade, Sanders, Boaventura, & Patchineelam, 2012). The Health Ministry published the National Relations of Medicinal Plants of Interest to SUS (RENISUS) aiming at the appropriate and safe use of

medicinal plants (Ministério da Saúde, 2018). Among these plants is the Zingiber officinale, popularly known as ginger, belongs to the Zingiber gender, which contains over 85 species.

Original from south Asia, ginger is now spread all over the world. Its global spreading, along the centuries, can be explained considering their medicinal properties or its use in different foods. In Asian, European and American cuisine, ginger is present in the preparation of dressings for meat and fish, of candies and of some alcoholic drinks (Júnior & Lemos, 2014).

Studies show that ginger acts in inflammatory processes, its components having immunomodulation and anti-swelling effects (Vieira et al., 2014). It even has satisfactory effects in the treatment of specific diseases such as type II diabetes, in which the phenolic compounds degrade the excess of free radicals generated in patients, thus reducing the hyperglycemic state (Lima, Silva, Cavalcante, & Campos, 2014). Ginger is also reported to have antimicrobial, antioxidant and antiemetic activity for the treatment of flus and colds, besides being diuretic and improving digestive functions (Foster & Chongxi, 1992; Ghosh, Banerjee, Millick, & Banerjee, 2011; Júnior & Lemos, 2014).

The concentrations used in food and medicinal administration of any plant must have necessary attention, including those with already elevated benefits, since all substances can be considered toxic agents depending on the conditions of exposure, dose administrated, time and frequency of exposure and way of administration (Oga, Camargo, & Batistuzzo, 2014). On the other side, the investigation of vegetables has grown, their extracts and chemical compounds isolated from the ones which have cytotoxic effect against cancer cells, with or without side effects to normal and healthy cells (Gomes, Priolli, Tralhão, & Botelho, 2013).

Among the techniques of analysis of toxicity of substances, the one that uses the vegetable Allium cepa L. works as a bio indicator ideal to the initial evaluations of cytotoxicity of medicinal plants, due to its low cost, reliability and agreement with other tests, assisting studies of reduction of damage to human health (Bagatini, Silva, & Tedesco, 2007). The in vitro tests-systems, used a culture of tumor cells, such as those from a human kidney, are especially used for tracking and generating wider toxicological profiles (Eisenbrand et al., 2002). These have contributed with identifying a great number of agents which can be potentially capable of reducing the risk of cancer development (De Flora & Fergurson, 2005).

Acknowledging this potential, it is necessary to know more about ginger's antioxidant, chemical characteristics, bioactive compounds, cytotoxic activity, mutagenic and antitumor activity, to serve as base to safe consumption and, so far, with beneficial properties to health.

Material and methods

Sampling

Ginger's rhizomes were bought at a local fair in Apucaraca-PR. They were sliced and dried in greenhouses with circulating air at temperatures 40, 60, and 80°C. After drying, the rhizomes were crushed to make powder and were stored in a fridge for future analysis.

Preparation of the extracts

The extracts of dry rhizomes at the three temperatures were prepared with ethanol:water (EtOH) 70:30 (v:v), ethanol:water 95:5 (v:v), methanol:water (MeOH) 70:30 (v:v) and methanol:water 95:5 (v:v), using 100 mL of solvent extractor with 1.0 g of ginger rhizome powder in magnetic agitation while sheltered from light. After four hours of agitation, the extracts were filtered into a volumetric balloon of 100 mL, the volume was adjusted with the appropriate solvent and stored under refrigeration in an amber bottle for future analysis. The extracts were prepared twice.

Determination of anthocyanins and yellow flavonoids

The determination of anthocyanins and yellow flavonoids of the dry rhizomes at the three temperatures were made by extracting the acid ethanol with hydrochloric acid, and the reading of the absorbance was done in an Agilent Tecnologies spectrophotometer, Cary 60 UV-VIS model, 535 nm for anthocyanins and 374 nm for yellow flavonoids (Francis, 1982). The results were expressed in mg for 100 grams.

Determination of ascorbic acid

The amount of ascorbic acid of the dry rhizomes at the three temperatures was determined by the extraction of oxalic acid and further titration with 2,6-dichlorophenolindophenol (Horwitz, 2000).

Determination of total phenols

The total phenolic compounds of the four extracts of dry ginger at the three temperatures, at concentration of 10,000 μg mL^{-1}, were determined by using the reagent Folin-Ciocalteau (Swain & Hills, 1959). The reading of absorbance was made using a 740 nm in an Agilent Tecnologies spectrophotometer, Cary 60 UV-VIS model. The standard curve of gallic acid (y = 0.003x + 0.026; R2 = 0.990) at concentrations of 350, 250, 200, 150, 100, 50, and 10 μg mL^{-1} was built and the results were expressed in EAG mg for 100 grams of dry ginger, where EAG represents the equivalent in gallic acid.

Determination of total flavonoids

The total flavonoids of the four extracts of dry ginger at the three temperatures, at concentration of 10,000 μg mL^{-1} were determined by the methodology that uses ethanol solution of aluminum chloride 5% (Woisky & Salatino, 1998). The readings were made at 425 nm in an Agilent Technologies spectrophotometer, Cary 60 UV-VIS model. The standard curve (y = 0.001x – 0.013; R2 = 0.994) at concentrations of 25, 50, 75, 100, 125, 150, and 200 μg mL^{-1} was prepared in a methane routine solution. The results were expressed in mg of routine 100 g^{-1} of dry ginger.

DPPH radical scavenging activity

The determination of antioxidant activity of the four extract of dry ginger at the three temperatures, at concentrations of 10,000 μg mL^{-1} and 1,000 μg mL^{-1} was done by monitoring the consumption of DPPH free radical in the samples, through the measure of decrease of absorbance, during 30 minutes, in a Agilent Technologies spectrophotometer, Cary 60 UV-VIS model at 517 nm (Brand-Williams, Cuvelier, & Berset, 1995). The antioxidant activity was expressed in a percentage of inhibition of control, according to the Equation 1, in which (Ac) represents the absorbance of control, (Ab) the absorbance of the white in the sample and (Aa) represents the absorbance of the sample. The pattern BHT (butyl-hydroxyl-toluene) and ascorbic acid were used at a concentration of 100 μg mL^{-1}.

$$AA\% = Ac - (Aa - Ab)/\mathrm{Ac} * 100$$

(1)

ABTS radical cation scavenging

The antioxidant activity in the four extracts of dry ginger, at concentrations of 10,000 μg mL^{-1} and 1,000 μg mL^{-1}, was made by the measure of inhibition of ABTS radical cation after 6 minutes of reaction (Rufino et al., 2007). The reading of absorbance was made in an Agilent Technologies spectrophotometer, model Cary 60 UV-VIS at 734 nm. The antioxidant activity was expressed in percentage of control inhibition, according to Equation 1. The pattern BHT (butyl-hydroxyl-toluene) and ascorbic acid were used at a concentration of 100 μg mL^{-1} .

Statistical analysis

The statistical analyses were done by using Statistica® 8.0 (StatSoft, Inc.) software. The presented results, obtained by the Tukey test ($p < 0.05$), correspond average ± standard curve. All the analysis were tripled ($n = 6$).

Citotoxicity and mutagenicity with Allium cepa L.

The texts of citotoxicity and mutagenicity with Allium cepa L. were conducted by putting onion bulbs to root in flasks with mineral water at room temperature, aired and in the dark for five days, with a water change every day (Fiskesjö, 1985).

After their growth, the onion roots were exposed to five different concentrations of alcoholic extract (EtOH 70:30) of dry ginger at 80°C: 1, 5, 10, 25, and 50%, diluted in mineral water. The roots were collected at 0 hour (control), after 24 hours of exposure to different control solution or treatment, and after 24 hours of exposure to mineral water (recovery) again, to avoid the recovery of any damage caused. The roots collected were exposed to a fixing solution of methanol and acetic acid for 24 hours (3:1), then, they were washed twice with distilled water, they remained exposed to a solution of chlorine acid 1 N at 60°C for 10 minutes, they were washed again, and exposed to the reactive of Schiff for 45 minutes in the dark. The meristem of the roots was used to make blades for maceration of acetic orcein.

The blades were analyzed in a light microscope, with objective of 40x, the "blind" test, containing a thousand cells a bulb, totalizing five thousand cells for each time the sample (control, treatment and recovery), for the control and treatment groups. The cells were differed in each of the phase of mitotic division: interphase, prophase, metaphase, anaphase and telophase. For the evaluation of cytotoxicity, the Mitotic index (IM %) was determined: the reason between the number of cells by the total number of analyzed cells, multiplied by 100. For the determination of mutagenicity, the cells with structural changed were counted, with colchinicic or disorganized metaphases, multipolar anaphases and loose chromosomes, micronucleous and others, and the calculus of total and average numbers of the chromosomic alterations was done. The statistical analysis was done by the test of qui-squared ($\alpha = 0.05$, $n = 5$).

Citotoxicity with tumoral cells from a human kidney

For the test of citotoxicity/antitumoral activity, the MTT test was done (Mosmann, 1983). Cell culture blades from 96 wells were used, where each well was seeded 0.5×10^4 tumor cells from human kidneys, 786-

O line, with the exception of control wells with no cells (blank). After stabilizing, the environment of culture of disposed of and it was added 100 µL to the negative control (CO-), positive control (CO+) groups with cytotoxic methyl methanesulfonate agent (MMS 50 µM), control solvent (CS) with the alcohol solution: water 70:30 (v:v) (150 µL mL^{-1}) and treatment with the various concentrations of alcoholic extract (EtOH 70:30) of dry ginger at 80oC (5, 25, 50, 75, and 150 µL mL^{-1}). After 24 or 48 hours of incubation, the culture was substituted by 100 µL of culture environment, with added MTT (0.2 mg mL^{-1}). The blades were incubated for over 4 hours prior to the disposal of the one containing MTT, followed by the addition of 100 µL of dimethylsulfoxide (DMSO), for the solution of formazan crystals. The reading of absorbance was done by a micro-plaques (Labtech) at 550 nm. The experiments were done in three different stages and the statistical analysis was made by Turkey test ($n = 3$, $p < 0.05$). The cell viability was esteemed based on the absorbance of the control according to the calculus of Equation 2.

$$VC = \left(\frac{Abs_{tratamento}}{Abs_{controle}}\right) * 100 \quad (2)$$

Results and discussion

The drying of natural products is importance since the humidity allows the proliferation of microorganism and enzymatic reactions. Most bioactive compounds present are sensible to temperature, degrading itself, so it is important to analyze the way the drying at different temperatures affects these compounds. The drying of ginger rhizomes was done at the temperatures of 40, 60 and 80ºC, with performance of 13.43, 15.22, and 15.29%, respectively, and with drying times of 26, 15, and 7 hours.

The ascorbic acid (vitamin C) is a hydro soluble antioxidant with great importance to human nutrition. The content of ascorbic acid varies a lot in food, being sensitive to heat and oxidation (Ceslestino, 2010; Ferreira, Fernandes, Fonte, Rodrigues, & Silva, 2010). In this study, the amount of ascorbic acid determined for dry rhizomes at temperatures of 40 and 60ºC were 75.19 ± 2.33 mg 100 g-1 and 78.33 ± 2.70 mg 100 g^{-1} for 80ºC. This way, ginger is classified as an average source of vitamin C at the drying temperatures studied (Silva, Martins, & De Deus, 2009).

The content of total phenolic compounds of the prepared extracts with dehydrated ginger at three temperatures and extracted with different solvents is presented in Table 1.

TABLE 1.
Results of total phenolic compounds in mg EAG 100 g^{-1}, sample for different extracts of ginger.

Solvent	40°C	60°C	80°C
EtOH 70:30	793.57 ± 19.40^{d}	958.97 ± 13.83$^{b, c}$	3,692.62 ± 47.67^{a}
EtOH 95:5	950.36 ± 28.45^{c}	833.96 ± 63.67^{c}	1,067.32 ± 38.36^{c}
MeOH 70:30	2,788.51 ± 41.15^{a}	2,808.79 ± 49.77^{a}	4,200.14 ± 293.66^{a}
MeOH 95:5	1,312.81 ± 19.37^{b}	1,104.02 ± 8.93^{b}	1,692.51 ± 50.10^{b}

Data represent the mean ± standard deviation of the samples (analyzed in triplicate). In the same column, the values with different letters are significantly different ($p < 0.05$).

The dada shows the content of total phenolic compounds of ginger is high, being the extracts MeOH 70:30 and EtOH 70:00, dried at 80ºC, showed the highest content, nor presenting significant differences (p

< 0.05). To the dryings of 40 and 60°C, the best solvent for extraction of phenolic compounds was MeOH 70:30. Our study demonstrates the influence of drying temperature for ginger. The solubility of phenolic compounds varies according to the polarity of the used solvent, the degree of polymerization of phenols and its interaction other sample constitutes (Naczk & Shahidi, 2004). In a similar study with jiló (Solanum gilo), it was observed the influence of the drying temperature and of the extracting solvent to the phenolic compounds (da Silva, Suzuki, Canesin & Tonin, 2017).

The phenolic compounds were intimately connected to the antioxidant action of natural products. Factures such as maturation, species, cultivation method, geographical origin, harvest conditions, storing and processing, can also influence the content of such compounds (Silva, Moreira, Melo, & Lima, 2012).

Table 2 presents the content of total flavonoids for different extracts of dehydrated ginger. The solvent which extracted the highest content of total flavonoids for ginger was MeOH 95:5 at 40°C. When analyzing the content of flavonoids for the other temperatures, comparing the extracting solvent, it is observed that the solvents MeOH 95:5 and EtOH 95:5 were the most effective extractors at 60°C, while the four solvents did not show significant different between them at 80oC, demonstration the same capacity of extracting this class of compounds. The increase in temperature negatively influenced the amount of flavonoids, probably because of its instability to hear, and methanol was more effective than ethanol in its extraction.

The anthocyanins play many roles in the organism, such as antioxidant action, protection from light, defense mechanism and biological function. Many factors influence the stability of anthocyanins, such as pH, temperature, presence of oxygen and light (Lopes, Xavier, Quadri, & Quadri, 2007). Table 3 presents the content of yellow flavonoids and anthocyanins obtained in mg 100 g^{-1} for dehydrated ginger.

TABLE 2

Results of total flavonoids present in dry ginger extract in mg of rutin 100 g^{-1}.

Solvent	40°C	60°C	80°C
EtOH 70:30	1,056.78 ± 64.63^{c}	743.14 ± 22.82^{c}	1,261.31 ± 45.43^{a}
EtOH 95:5	1,875.21 ± 68.26^{b}	1,190.36 ± 19.33^{a}	1,394.36 ± 66.44^{a}
MeOH 70:30	1,948.37 ± 14.84^{b}	1,024.68 ± 23.08^{b}	1,271.57 ± 155.23^{a}
MeOH 95:5	2,252.69 ± 38.93^{a}	1,204.01 ± 60.20^{a,c}	1,304.89 ± 11.49^{a}

Data represent the mean ± standard deviation of the samples (analyzed in triplicate). In the same column, the values with different letters are significantly different (p < 0.05).

The numbers found in the present study demonstrated the degradation of anthocyanins and the reduction of the content of yellow flavonoids as temperature is increased. The values of yellow flavonoids found for dry ginger were high when compared to tropical fruits such as acerola cherry (98.05 ± 0.19 mg 100 g^{-1}), cashew (44.91 ± 4.43 mg 100 g^{-1}) and papaya (97.30 ± 0.96 mg 100 g^{-1}), presenting values similar to the ones of pitanga fruit when dried at 40°C (207.87 ± 19.43 mg 100 g^{-1}) (Silva et al., 2014).

TABLE 3.

Content of anthocyanins and yellow flavonoids in mg 100 g^{-1} of dry ginger.

T (°C)	Anthocyanins	yellow flavonoids
40	10.90 ± 0.13^{a}	200.15 ± 0.82^{a}
60	6.96 ± 0.30^{b}	144.53 ± 0.06^{c}
80	7.31 ± 0.23^{b}	153.43 ± 0.14^{b}

Data represent the mean ± standard deviation of the samples (analyzed in triplicate). In the same column, the values with different letters are significantly different (p < 0.05).

Table 4 presents the values of percentage of radical DPPH scavenging for the extracts of ginger at concentration of 10,000 μg mL^{-1} and 1,000 μg mL^{-1} at the different drying temperatures.

TABLE 4.
Percentage of radical DPPH inhibition of ginger extracts in concentration of 10,000 μg mL^{-1} and 1,000 μg mL^{-1} after 30 minutes of reaction.

Solvent	40°C	60°C	80°C
10,000 μg mL^{-1}			
EtOH 70:30	86.82 ± 0.06^{b}	92.21 ± 0.12^{a}	91.29 ± 0.12^{a}
EtOH 95:5	93.43 ± 0.36^{a}	93.80 ± 0.01^{a}	93.84 ± 0.16^{a}
MeOH 70:30	88.45 ± 1.18^{b}	85.29 ± 0.62^{b}	91.88 ± 0.39^{a}
MeOH 95:5	92.62 ± 0.49^{a}	96.45 ± 1.46^{a}	90.45 ± 0.79^{a}
1,000 μg mL^{-1}			
EtOH 70:30	40.13 ± 4.11^{d}	44.28 ± 1.95^{d}	74.03 ± 3.12^{b}
EtOH 95:5	46.21 ± 0.43^{d}	34.84 ± 0.78^{d}	55.64 ± 0.86^{d}
MeOH 70:30	72.41 ± 1.41^{c}	67.18 ± 0.58^{c}	65.35 ± 0.43^{c}
MeOH 95:5	68.56 ± 0.56^{c}	71.39 ± 0.63^{c}	66.33 ± 0.26^{c}

Data represent the mean ± standard deviation of the samples (analyzed in triplicate). In the same column, the values with different letters are significantly different ($p < 0.05$).

The ginger extracts at concentration of 10,000 μg mL^{-1} demonstrated a high antioxidant power in all temperatures, with sequestration percentage of the DPPH radical superior to 85% in 30 minutes. In concentration 1,000 μg mL-1, the most active extracts were MeOH 70:30 and MeOH 95:5 for the dryings at 40oC and 6oC and EtOH 70:30 for the drying at 80oC, not presenting significant differences (analysis not presented in the table), a positive correlation with the content of the bioactive compounds and antioxidant activity being observed.

The kinetic behavior of the antioxidant compounds can be classified according to the consumption time of 50% of the DPPH radical (TC50). When TC50 is lower than 5 minutes, the kinetic is classified as fast; if TC50 is between 5 to 30 minutes, it is intermediate; and it is slow when TC50 is longer than 30 minutes (Sanchéz-Moreno, Larrauri, & Saura-Calixto, 1998). The BHT pattern (100 μg mL^{-1}) presented slow kinetic consumption of DPPH radical (32.2 ± 0.18% in 30 minutes), while the ascorbic acid presents fast kinetic, consuming 94.7 ± 1.24% of radical immediately. All extracts prepared at concentration of 10,000 μg mL^{-1} presents fast kinetic, consuming more than 80% of radical in less than 5 minutes. These demonstrate greater antioxidant power than the BHT pattern and similar efficiency to ascorbic acid.

This way, dry ginger, at distinct temperatures, presents a good sequestration potential of DPPH radical. Its antioxidant potential can be attributed to the presence of vitamin C and phenolic compounds, such as flavonoids and anthocyanins, especially gingerol and shogaol (Kikuzari & Nikatani, 1993).

Table 5 shows the results in antioxidant activity for ginger determined by ABTS+ method.

TABLE 5.
Percentage of ABTS radical inhibition of the different ginger extracts for the concentration of 10,000 μg mL^{-1} and 1,000 μg mL^{-1} after 6 minutes of reaction.

Solvent	40°C	60°C	80°C
10,000 μg mL^{-1}			
EtOH 70:30	43.21 ± 0.13^{b}	36.16 ± 0.44^{c}	69.97 ± 0.78^{a}
EtOH 95:5	36.91 ± 0.71^{c}	28.67 ± 1.62^{d}	47.22 ± 3.75^{b}
MeOH 70:30	46.85 ± 0.38^{b}	40.66 ± 0.22$^{b, c}$	74.70 ± 2.16^{a}
MeOH 95:5	55.71 ± 0.29^{a}	55.40 ± 1.30^{a}	51.88 ± 1.22^{b}
1,000 μg mL^{-1}			
EtOH 70:30	5.87 ± 1.50^{e}	14.70 ± 1.98^{e}	9.30 ± 1.66^{d}
EtOH 95:5	5.80 ± 4.01^{e}	11.05 ± 1.43^{e}	11.08 ± 0.34^{d}
MeOH 70:30	10.82 ± 0.38^{d}	13.16 ± 0.69^{e}	17.51 ± 2.83^{c}
MeOH 95:5	4.74 ± 0.50^{e}	12.10 ± 0.52^{e}	17.58 ± 0.74^{c}

Data represent the mean ± standard deviation of the samples (analyzed in triplicate). In the same column, the values with different letters are significantly different ($p < 0.05$).

At concentrations of 10,000 μg mL^{-1}, the extract from the dryings at 40 and 60oC which presented highest antioxidant potential when in contact with ABTS radical was MeOH 95:5. At temperatures of 80oC, the solvents MeOH and EtOH 70:30 presented its highest radical sequestration potential, comparing solvents and drying temperatures. In concentration 1,000 μg mL^{-1} there was no significant difference for the extracts at 40 and 60°C, and, for 80°C, MeOH 70:30 and 95:5 were the ones which present highest percentage of scavenging of ABTS.

For this method, a positive relation between antioxidant activities and the content of phenolic compounds and flavonoids was observed. Previous studies with ginger report extraction by supercritical fluid and the preparation of extracts with high concentration levels of phenolic compounds and compounds capable of reducing free radicals (Justo, Moraes, Barreto, Mercadante, & Rosa, 2008).

Based on all the results obtained, it can be concluded that dry ginger at 80oC and its extracts with EtOH and MeOH 70:30 were the ones with had, in general, the best content of bioactive compounds and of antioxidant activity. Considering that ethanol is a solvent that does not leave toxic residue, differently from methanol, and that it is considered safe for human health, the studies for evaluation of the biological activity of ginger were made with EtOH 70:30 of dry ginger at 80°C.

The data from the cytotoxicity test with *Allium cepa* L. (Figure 1) indicate that the different concentrations of ginger extract were not cytotoxic for onion meristem cells. None of the concentrations of the extract presents statistical different in relation to the negative control and the solvent, in the different analyzed times, not even when compared to the control of its own bulb at 0 hour. Despite that, a decrease in the mitotic index in 24 hours (treatment with ginger extract) compared to collection time of 0 hours (control). The work of Carvalho and Rodrigues (2011), on the other hand, indicate cytotoxic effect of ginger tea, in concentrations of 10, 25, 50, and 100 g L^{-1} of water, for the meristem cells of *A. cepa*, interrupting the cellular cycle and causing the process of division in the interphase to stop. The concentration of 10 g L^{-1} is the same as studied in the present study, but with ethanol with solvent, what can result in a higher presence of bioactive and antioxidant compounds, as demonstrated in performed analysis, and, as a consequence, the absence of cytotoxicity.

However, the data in Figure 1 show that the increase in concentration of ethanol extract of ginger (from 1% to 50%) a small decrease of the mitotic percentage decreased. Silva, Silva & Pires (2017) also observed the increase in concentrations of the infusions in natura of *Zingiber officinale* (6.6, 20, 33.3, 46.6, and 60 g L-1 of water) presented a tendency of reduction of mitotic index of *A. cepa*.

The test with *A. cepa* has been widely used to analyze cytotoxicity and mutagenicity of medicinal plants, besides being great at monitoring environmental pollution and evaluation of potential of many chemical compounds (Ma et al., 1995; Debnath, Mondal, Hajra, Das, & Mondal, 2018).

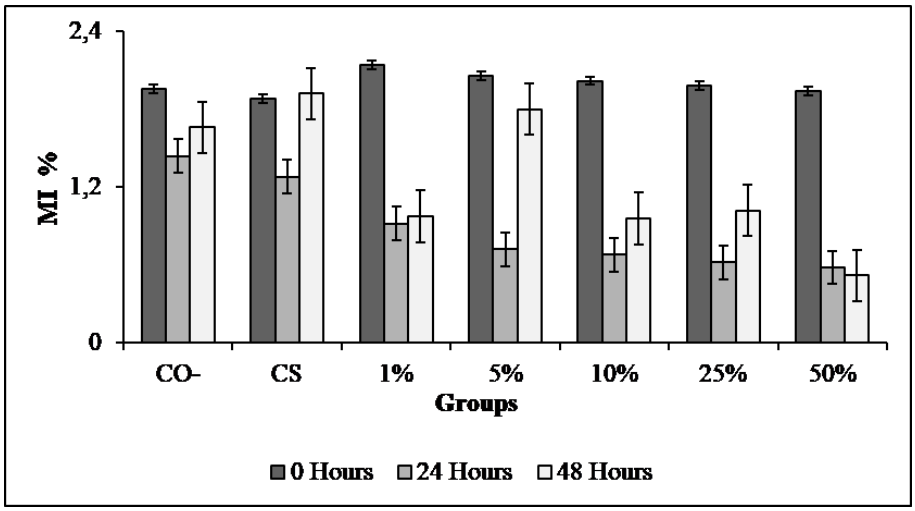

FIGURE 1.
Percentage of mitotic index (IM) for the negative control group (CO-), solvent control (CS – 70 alcohol: 30 water) and groups treated with different concentrations of ethanolic extracts of ginger (70:30) (1 to 50%) in times 0 hour (control), 24 hours (treatment) and 48 hours (recovery).

The methods of evaluation of chromosomic alterations using onion roots as test organism is valid by the International Program of Chemical Security (IPCS, OMS) and the United Nations Environmental Program (UNEP) as an efficient test to study a genotoxicity of substances (Cabrera & Rodriguez, 1999; Modlitbová et al., 2018).

In the present study, some chromosomic alterations were identified in the mitotic anaphase and interphase of treated groups with different concentrations of ginger extract (Table 6), but their average numbers were not statistically different from the ones found in control 0 hours of its own bulb and in negative control of the experiment, showing that ginger was not mutagenic. These results diverge from the ones found by Carvalho and Rodrigues (2011) and Silva et al. (2017) which showed the increase in the concentration of the watery extract of ginger favored the appearance of anomalies, such as micronucleous and sprouts, responsible for the genotoxic effect of ginger infusions for *A. cepa*. Again, the results of the present study can be justified by the chemical characteristics of the ethanol extract.

TABLE 6.
Total, averages and types of chromosomic alterations found for the groups negative control (CO-) and solvent (CS) and treated with different concentrations of ethanolic extract of ginger (70:30).

Treatments	Time the Sample	Phase of Mitotic Division and Chromosomic Alterations								Total of Alterations	Average Number of Alterations
		Interphase	Prophase	Metaphase		Anaphase			Telophase		
		MN	DS	MC	DS	LC	MP	DS	P		
CO-	0h	-	-	-	-	1	-	-	-	1	0.125
	24h	1	-	-	-	1	-	-	-	2	0.250
	48h	-	-	-	-	-	-	-	-	-	0
CS	0h	-	-	-	-	1	-	-	-	1	0.125
	24h	-	-	-	-	2	-	-	-	2	0.250
	48h	-	-	-	-	1	-	-	-	1	0.125
1%	0h	-	-	-	-	-	-	-	-	-	0
	24h	-	-	-	-	-	-	-	-	-	0
	48h	1	-	-	-	-	-	-	-	1	0.125
5%	0h	-	-	-	-	-	-	-	-	-	0
	24h	-	-	-	-	1	-	-	-	1	0.125
	48h	-	-	-	-	1	-	-	-	1	0.125
10%	0h	-	-	-	-	-	-	-	-	-	0
	24h	-	-	-	-	2	-	-	-	2	0.250
	48h	-	-	-	-	-	-	-	-	-	0
25%	0h	-	-	-	-	-	-	-	-	-	0
	24h	-	-	-	-	-	-	-	-	-	0
	48h	1	-	-	-	1	-	-	-	2	0.250
50%	0h	-	-	-	-	-	-	-	-	-	0
	24h	-	-	-	-	-	-	-	-	-	0
	48h	-	-	-	-	1	-	-	-	1	0.125

MN= Micronucleous; DS= Disorganized; MC: Metaphase-Colchicinic; LC: Loose Chromosome; MP: Multipolar; P: Bridge.

On the other hand, the data of the cytotoxicity/antitumor activity test with cancerous cells from a human kidney (Figure 2) show that in 24 hours, all concentrations of the ethanol extract of ginger presented cytotoxic potential. Besides, a dose-independent effect can be observed, in which the greater the concentration of the extract, the greater the cytotoxic effect and the lesser the cell feasibility, the highest concentrations (> 25 μL mL^{-1}) presenting lower cell feasibility than 52% (Table 7) and were statistically different from the greatest concentrations (5 μL mL^{-1}), which presented cell feasibility of 83.12%.

In 48 hours, all concentrations of ginger extract continued presenting cytotoxic effect (Figure 2), still dose-dependent and with greater effectiveness at higher concentrations, which presented viability cellular lower than 30% (Table 7). However, in this case, due to the similarity of absorbance and cell feasibility of higher concentrations with the absorbance of the control solvent, it is possible that this effect is due to the solvent and not to the compounds present in ginger.

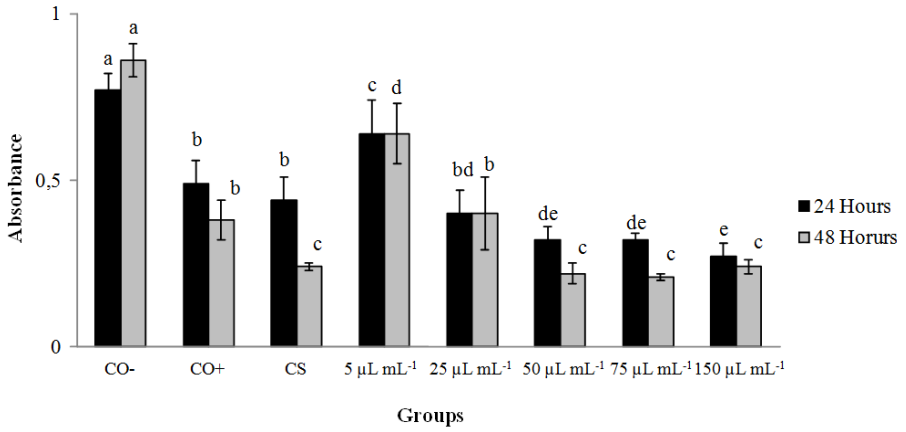

FIGURE 2.
Average absorbance and standard-deviation for tumor cells from human kidney treated with the different concentrations of ginger ethanol extract (70:30), incubated for 24 and 48 hours.

TABLE 7.
Percentage of the viability of tumor cells (VC) from a human kidney, treated with ethanolic extract of ginger (70:30), for 24 and 48 hours, according to MTT test.

Groups		VC (%) 24h	48h
CO-		100.00^{a}	100.00^{a}
CO+		63.64^{b}	44.19^{b}
CS		57.14^{b}	27.91^{c}
Ginger extract	5 µL mL^{-1}	83.12^{c}	74.42^{d}
	25 µL mL^{-1}	51.95^{bd}	46.51^{b}
	50 µL mL^{-1}	41.56^{de}	25.58^{c}
	75 µL mL^{-1}	41.56^{de}	24.42^{c}
	150 µL mL^{-1}	35.06^{e}	27.91^{c}

Groups: CO-: Negative Control; CO+: Positive Control; CS: Control Solvent; 0.5x104 cells per well.
Means followed by the same letter do not differ statistically from each other by the Tukey test ($p < 0.05$).

In the study of Amri and Touil-Boukoffa (2016), with the watery extract of ginger and 6-gingerol, the feasibility of protozoans in vitro was also reduced, being related to the concentration of the studied compounds and to the time of exposure, as occurred in the present study, in which the higher the concentration and time of exposure, the smaller the cell feasibility.

According to Barreto, Toscano, and Fortes (2012) in a study on the effects of ginger in oncologic patients treated with chemotherapy, 6-gingerol, one of the main components of ginger, is capable, among other things, to inhibit tumor production and to induce the production of the factor of tumor necrosis (TNF-α) which may have occurred in the present study and resulted in the antitumor activity of ginger alcoholic extract.

However, the Prazeres and Vicentini (2017) study, which tested the compounds Zingerone from ginger, using cancer cells from human breasts and the MTT test, the compound did not show cytotoxic effect in any of the evaluated concentrations (5, 10, 20, 30, 40, 50, 100, 200, 300, and 500 µM) in none of the times (24, 48, and 72 hours), with viability cellular higher to 80%. These data show that different types of cells can have different cytotoxic responses and, possibly, that the antitumor effect of ginger must exist due to the mixture

of bioactive compounds, such as phenolic compounds, anthocyanins, flavonoids, vitamin C, and antioxidant activity, which were present in the extract studied in this study.

Conclusion

Zingiber officinale presenting significant values of bioactive compounds such as phenolics compounds, total flavonoids, yellow flavonoids and anthocyanins. The extracts with higher antioxidant power are EtOH 70:30 for dry rhizome at 80ºC and MeOH 95:5 for rhizomes dried at 40ºC. The EtOH 70:30 extract for dry rhizome at 80oC was not cytotoxic or mutagenic for the meristemic cells of Allium cepa L., in its tested concentrations. More relevantly, it presented a cytotoxic effect, in all tested concentrations, for tumor cells from a human kidney, indicating that it is possible to reduce the cellular proliferation of this type of cell and to be antitumor.

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