Original Article

GENOTOXICITY AND ANTIOXIDANT ACTIVITY OF SPICES AND HERBS USED IN BRAZILIAN CUISINE

GENOTOXICIDADE E ATIVIDADE ANTIOXIDANTE DE ESPECIARIAS E ERVAS UTILIZADAS NA CULINÁRIA BRASILEIRA

Regildo Marcio Gonçalves SILVA[1]; Ana Carolina Monetta CARVALHO[2]; Larissa Silva MATIOLLI[3]; Célia Cristina Malagutti FIGUEIREDO[4]; Amanda Costa GOMES[5]; Paulo Cesar FERREIRA[5]; Luciana Pereira SILVA[6]

1. Professor Doutor. regildos@yahoo.com.br, 2. Mestre Bióloga, 3. Mestre Naturóloga, 4. Nutricionista, 5. Mestres Farmacêuticos, Universidade Estadual Paulista - UNESP, Departamento de Biotecnologia - Laboratório de Fitoterápicos e Produtos Naturais, Faculdade de Ciências e Letras de Assis, Assis, São Paulo, Brasil; 6. Pós-Doutoranda, Universidade Estadual do Norte do Paraná – UENP, Programa de Pós-graduação em Agronomia, Campus Bandeirantes - Paraná.

ABSTRACT: Spices are natural plant products, have been used not only as flavoring and coloring agents, but also as food preservatives and folk medicines throughout the world for thousands of years. Many spices also have been recognized by having both digestive stimulant and carminative actions and also antimicrobial, anti-inflammatory, anti-mutagenic and anti-carcinogenic potential. Antioxidant and genotoxic potential of species commonly used in Brazil was evaluated. The antioxidant activity was evaluated using different methods, including DPPH radical scavenging activity, ferric reducing power (FRAP), iron ion chelating power, inhibition of lipid peroxidation (TBARS), NO radical scavenging, and oxidative hemolysis inhibition. Furthermore, the antigenotoxic activity was evaluated through mitotic index and chromosome aberration in Allium cepa roots. Quantification of total phenols and flavonoids carried out. The results with the *Ocimum basilicum* spices in the DPPH test showed activity (82.01%), FRAP (321.12 uM ET and iron chelating activity (94.18) and for the *Cinnamomum zeylanicum* spice in the TBARS test (18.52%) evaluated by different methods and mechanisms of inactivation of free radicals and according to the evaluation of genotoxicity by the *Allium cepa* test the spices do not present genotoxic effects.

KEYWORDS: Antioxidant activity. Genotoxicity *Cinnamomum zeylanicum*. *Origanum vulgare*. *Piper nigrum*. *Zingiber officinale*. *Ocimum basilicum.*

INTRODUCTION

Brazil has a very original culinary. Over the centuries, Brazilian population has transformed European culinary, mainly from Portugal, Spain, Italy and Germany, adding spices from Asia and ingredients from native Indian and African culinary (RODRIGUES et al., 2007; MENDES et al., 2015). They are often defined as aromatic, dried plant substances applied to foods primarily for flavoring and coloring, rather than for nutritional values. In addition to true spices, such as black pepper, the term spice is often used in a wider context to include dried herbs, aromatic seeds and dried vegetable (PAPAGEORGIOU et al, 2008; FATIHA et al, 2015). Spices may include various anatomical parts of plants (e.g. roots, rhizomes, leaves, flowers, fruits, seeds, corms, bark) which are characterized by specific sensory values and exert beneficial effects on digestive processes (TIERAONA LOW DOG, 2006). Substances that determine such properties of spices include volatile oils, essential acids, alkaloids, resins, sulfur compounds and flavonoids (HURTADO-FERNÁNDEZ et al, 2010). In addition, these compounds also contain the vitamins A, E and C, which contribute to antioxidant capacity (HELMJA et al, 2007, EMBUSCADO, 2015).

Antioxidants are compounds that protect biological systems against the harmful effects of free radicals. Radicals that are not properly neutralized can initiate the detrimental process of oxidative stress in cells and tissues which may lead to several damage to health including neurodegenerative and cardiovascular diseases, cancer and premature aging (LU et al., 2011; ABBASI et al., 2015; FATIHA et al., 2015). The acting of antioxidants can be as interceptors of free radicals, sequestering agents of metals and inhibitors of oxidative enzymes (HURTADO-FERNÁNDEZ et al, 2010; HELMJA et al, 2007). There are several synthetic antioxidants now available but they are costly and many of them are associated with multiple side effects such as anorexia, nausea and diarrhea. In view of the former, there has been growing interest in finding natural antioxidants that may help the organism to prevent the oxidation of cellular, oxidizable substrates or even retard the progression of diseases induced by free radicals (KAEFER AND MILNER,

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2008; JUNGBAUER and MEDJAKOVIC, 2012; VIUDA-MARTOS et al., 2011).

Added to this, recent investigations have revealed that many plants used as spice in food may have cytotoxic, mutagenic and genotoxic properties. Therefore, studies on the genotoxicity and cytotoxicity of herbs and spices frequently used by people are need to identify which present genotoxic, mutagenic and carcinogenic risks.

High quality spices, such as saffron, vanilla and cinnamon, belong to the most expensive products because of its rare sources, unusual production conditions or complicated crop (REINHOLDS et al, 2015). Spices are natural plant products, which have been used not only as flavoring and coloring agents, but also as food preservatives and folk medicines throughout the world for thousands of years. Many spices also have been recognized by having both digestive stimulant and carminative actions and also antimicrobial, anti-inflammatory, anti-mutagenic and anti-carcinogenic potential (LU et al., 2011; ABBASI et al., 2015; FATIHA et al., 2015). Folk medicine is used worldwide and having great economic value in the 21st century in both developed and developing countries. Plants are rich in active compounds, thus knowledge on plant diversity of an area and the medicinal use of those plants by local people is of prime importance for development of those species considered effective in the treatment of various ailments. Among the species used as flavoring agents in Brazilian dishes, the most common ones are *C. zeylanicum* Breyn (cinnamon), *O. vulgare* L. (oregano), *P. nigrum* L. (black pepper), *Z. officinale* Roscoe (ginger) and *O. basilicum* L. (sweet basil) (SILAMBARASAN; AYYANAR, 2015). Information about the therapeutic potential of these plants, based in several studies, is given on table 1. Considering the variety of spice plants used in Brazilian culinary, the aim of this study was to evaluate the antioxidant and genotoxic potential of species commonly used in Brazil.

Table 1. Therapeutic potential of the spice plants evaluated in this study.

Cientific name	Popular name	Family	Therapeutic potential
Cinnamomum zeylanicum	*Cinnamon*, Canela	Lauraceae	Antioxidant[a], fungicide[b], cicatrizant[c]
Origanum vulgare	Oregano	Lamiaceae	Antioxidant[d], Antihyperglycemic[e]
Piper nigrum	Black pepper	Piperaceae	Larvicide[f], antioxidant[g]
Zingiber officinale	Ginger	Zingiberaceae	Hypoglycemic[h], hypocholesterolemic[i], antihemetic[i], antiinflamatory[i], antitumoral[j], antirheumatic[k]
Ocimum basilicum	Sweet basil, basil, manjerição	Lamiaceae	Antimicrobial[l], Antioxidant[m],

a- Mathew and Abraham, 2006; b- Ranasinghe et al., 2002; c- Kamath et al., 2003; d- Ivanova et al., 2005; e- Lemhadri et al., 2004; g- Campos et al., 2009; h-Park et al, 2002; h- Gulçin, 2005; i- Baldreldin et al., 2007; j- Vimala et al., 1999; k- Srinivastava e Mustafa, 1992; l- Sartoratto et al., 2004; m- Javanmardi et al.,2003.

MATERIAL AND METHODS

Plant sample

The spice plants used in this study were obtained as bark (cinnamon), fresh leaves (oregano and basil), seeds (black pepper) and roots (ginger), purchased from local grocery stores in Assis/SP, Brazil.

Plant extracts

Extracts derived from fresh leaves were prepared as infusion with water (oregano and basil): 10mL boiling water was added each 1g of fresh leaves. Then, the extracts were filtered through a paper filter. Extracts derived from barks of cinnamon, seeds of black pepper and roots of ginger were prepared by decoction: to each 1g of barks, seeds or roots was added 10mL of boiling water, incubated for 5 min and then filtered on filter paper. The filtrates were frozen and lyophilized to obtainment of dry extracts. Then, they were diluted in different concentrations of ethanol.

Test of the antioxidant activity scavenging DPPH radical test

The antioxidant activity of the crude extract and fractions was determined by the ability of the H^+ donor to stabilize radical 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma, USA), according to the *in vitro* methodology proposed by Blois (1958).

The experiment was performed in triplicate using a solution comprised of 1 mL of acetate buffer (pH 5.5 and 100 mM), 1.25 mL of ethanol P.A., 250 µL of DPPH solution and 50 mL of samples. The extract or fraction reacted with DPPH radical for a period of 30 min under low light and was then subjected to a UV-Vis spectrophotometer (Femto-600 Plus) at an absorbance of 517 nm (BRAND-WILLIAMS et al., 1995). The calculation of antioxidant activity was performed according to the following formula: antioxidant activity (%) = [(control-sample)/control] x100. The antioxidant activity of the extracts can be determined by the degree of discoloration of the reagent after the 30 min required for the reaction to attain a plateau.

Ferric ion reducing antioxidant power (FRAP assay)

FRAP activity was measured according to the method described by Rufino et al. (2006). Briefly, acetate buffer (300mM, pH 3.6), TPTZ (2.4.6-tripyridyl-s-triazine) 10mM in 40mM HCl and $FeCl_3 \cdot 6H_2O$ (20mM) were mixed in the ratio of 10:1:1 (v/v/v) to obtain the FRAP reagent. The reagent (2.7mL), ultrapure water (270µL) and sample solutions (90µL) were mixed thoroughly. Next we kept it in a water bath (Cienlab® model EC 400/4) for 30 minutes at 37°C. The absorbance was measured at 593nm. A standard curve was prepared using Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic SIGMA®) (30-500µmol). The results were expressed in µMTrolox equivalent (TE) per gram of dry extract. All determinations were performed in triplicates.

Iron-ion-chelating activity

The iron-chelating ability of the extracts was estimated by the method of Dinis et al. (1994). Tree dilutions in methanol (250, 500 and 1000 µg mL^{-1}) were prepared from the dried extract. Briefly, 0.05 mL of each dilution was added to a 2.7 mL TRIS buffer (pH=7.4). Thereafter, 0.05 mL of 2 mM $FeCl_2$ was added and vortexed for 15 seconds. At 30 seconds, the reaction was initiated by the addition of 5mM ferrozine (0.2 mL), the mixture was shaken vigorously at Vortex (VelpScientifica,UE) for 10 seconds. After 1 minute beyond addition of $FeCl_2$ solution, the absorbance was measured spectrophotometrically at 562nm. The ability of extract to chelate the ferrous ion was calculated relatively to the control (consisting of TRIS buffer, iron and ferrozine only) using the formula: chelating activity (%) = 100 x $[(A_C A_S)/A_C]$, where A_C is the absorbance of the control, and A_S is the absorbance of the sample.

Nitric oxide scavenging

The nitric oxide (NO) scavenging assay was accomplished by using sodium nitroprusside (SHAHRIAR et al., 2013). This can be determined by the use of the Griess reaction. Two mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline PBS (pH 7.4) was mixed with 0.5 mL of methanolic extract at various concentrations (250, 500 and 1000 µg mL^{-1}) and incubated at 37 °C for 150 minutes. After incubation the mixture was maintained for 60 minutes at room temperature. The absorbance was measured at 540 nm. The percentage of inhibition was calculated according to the following equation referent to the sodium nitrite calibration curve (y=0.0052x+0.0349).

Lipid peroxidation assay (TBARS)

A modified thiobarbituric acid reactive species (TBARS) assay (AWAH et al., 2010), using egg yolk homogenates as lipid-rich media (RUBERTO; BARATTA, 2000), was performed to measure the formation of lipid peroxide. Malondialdehyde (MDA), a secondary end-product of the oxidation of polyunsaturated fatty acids that reacts with two molecules of thiobarbituric acid (TBA) to yield a pinkish red chromogenic with maximum absorption at 532 nm. Egg homogenate (500 µl) of 10%, v/v in phosphate buffered saline (pH 7.4) and 100 µl of sample (250, 500 and 1000 µg mL^{-1}) were added to 1mL of the hydrochloride 2.2"Azobis (2-amidinopropane) dihydrochloride (AAPH) (0.12M) to induce lipid peroxidation. The mixture was incubated for 30 minutes at 37ºC. After incubating and cooling at room temperature, 0.5mL of trichloroacetic acid (ATA) (15%) and 0.5mL of TBA (0.6%) were added. The mixture was incubated at 97ºC for 15 minutes. After incubation, centrifugation was performed with 1mL of n-butane (2783 rpm for 10 minutes). Then, the reading of the supernatant was performed at 532 nm. Inhibition (%) of lipid peroxidation was calculated using the following equation: Inhibitory activity (%) = $[(A_C - A_S)/A_C]$ x 100, where A_C is the absorbance of the control and A_S is the absorbance of the sample.

Inhibition oxidative hemolysis human erythrocyte

All the procedures described in the study protocol were approved by the Ethics Committee of the Universidade Estadual Paulista - UNESP and all subjects gave a written informed consent. For this study, blood was obtained from healthy donors who were non-smokers and not receiving any pharmacological treatment. Their blood was collected in tubes containing EDTA anticoagulant.

Every experiment was performed each day with the erythrocytes that came from the same donors.

Anticoagulated blood tubes were centrifuged 5 min at 2500 rpm, plasma and the white cell layer (buffy coat) were discarded after and the remaining erythrocytes were washed three times with phosphate *buffered* saline (PBS). An erythrocyte suspension was prepared at 10% concentration in PBS. AAPH (2,2′-azobis (2-amidinopropane) hydrochloride solution was prepared using the same buffer at the moment of its use, and also kept away from the light. The extract was dissolved in 50% stock-solution of propylene glycol, and later diluted in PBS at the time of use. The incubation of erythrocyte suspension with AAPH in the presence or absence of extracts at different concentrations was performed in a 37ºC water-bath with a gentle shaking motion and in the absence of light for 6h. Positive (100% hemolysis) and negative controls were incubated with and without AAPH, respectively, included in the assay to examine a protective effect of the extracts (YANG et al., 2006; BANERJEE et al., 2008).

Aliquots were removed every one hour, transferred to *eppendorf* tubes and centrifuged at 2500 rpm for 10 minutes and then spectrophotometric reading of supernatant was performed. Hemolysis was determined by reading the absorbance of hemoglobin at 540 nm. The test samples were done in triplicate for statistical analyzes and percentage hemolysis was calculated by the following equation: % Hemolysis = 100.[(A_S-A_{NC})/(A_{PC}-A_{NC})] where A_S is the absorbance of the sample, A_{NC} is the absorbance of the negative control and A_{PC} is the absorbance of the positive control.

Statistical analysis

The data was expressed as the mean±SD by measuring three independent replicates. Analysis of variance using one-way ANOVA followed by Tukey's test was performed to test the significance of differences between the means obtained among treatments at the $\alpha \leq 0.05$ level of significance using the software BioEstat version 5.0.

Quantification of total phenols and flavonoids

The total phenols and flavonoids were quantified with the extract diluted in ethanol at concentrations of 250, 500 and 1000 $\mu g.mL^{-1}$.The Folin-Ciocalteu method modified by Singleton and Rossi (1980) was performed to determine total phenols. For each 0.5 mL of extract at different concentrations, 5mL of distilled water and 0.25 mL of Folin-Ciocalteu reagent were added. After 3 minutes, 1mL of saturated Na_2CO_3 solution at 10% was added and the mixture was stored for 1 hour. The absorbance was measured at 725nm using a UV-Vis spectrophotometer (model: SP220, Biospectro, Brazil). All the tests were performed in triplicate and the results were expressed in mg of gallic acid per gram of extract.

Quantification of the extract's total flavonoids was performed by UV-Vis spectrophotometer, with the preparation of samples based on complexation of flavonoids with $AlCl_3$ as described by Zhishen; Mengcheng and Jianming (1999). An aliquot of 250 µL of each different concentration of extract was mixed with 1.25 mL of distilled water and 75 µL of $NaNO_2$ solution at 5%. After 6 minutes, 150 µL of $AlCl_3/H_2O$ solution at 10% was added. After 5 minutes, 0.5 mL of NaOH 1M solution was added and then the total volume was completed by adding 2.5 mL of distilled water. The samples were shaken in a vortex mixer and the absorbance was measured at 510 nm. All the tests were performed in triplicate and the results were expressed in mg of rutin per gram of extract.

Genotoxic activity Mitotic index and chromosome aberration in *Allium cepa* Roots

Onion bulbs (A. cepa L., 2n = 16) were obtained commercially in Assis, São Paulo, Brazil. They were cleaned and dried outer scales were removed, leaving the ring intact with primordial roots. The bulbs were used for the bioassay according to standard procedures (RANK; NIELSEN, 1993; CHAUHAN et al., 1999; BABATUNDE; BAKARE, 2006). For the growth of roots it was used a culture solution (Hoagland's solution). The bulbs were kept suspended in a 100mL becker leaving the ring of roots in contact with the solution, changed every 24 h for a period of 72 h, maintained at a photoperiod (18 h/6 h light/dark) and temperature (22 ± 2°C) in controlled chamber B.O.D. Bulbs with roots of approximately 2cm were used in the experiment.

To evaluate the mitotic index and induction of chromosomal aberrations (aberrant anaphase and telophase), six onion bulbs were exposed to each concentration of aqueous extract (0.1, 5 and 10 mg/mL) and ethanolic extract (50, 100 and 1000 µg mL^{-1}) of *B. trimera*. Mineral water and positive control solution of MMS (methyl methanesulfonate, Sigma-Aldrich ®) at 10 mg/L was used for the negative control, as described by Caritá and Marin-Morales (2008). At the end of 48h exposure and 24h recovery in culture solution, the roots of treated and control bulbs were cutted and fixed in ethanol: glacial acetic acid (3:1, v/v). These were hydrolyzed

in 1 N HCl at 60°C for 8 min, after which they were rinsed in distilled water. The roots were stained with acetic carmine for 10 minutes. The tips were removed and the roots carefully crushed between slide and coverslip were sealed as suggested by Grant (1982) and Akinboro and Bakare (2007). Five slides were prepared for each treatment and controls were analyzed at 1000× magnification. The mitotic index was calculated on the number of dividing cells per 1000 cells observed (FISKESJÖ, 1985; FISKESJÖ et al., 1997). The frequency was calculated based on the number of aberrant cells (telophases and anaphases) per total cells analyzed for each treatment and controls (BAKARE et al., 2000).

Root growth determination

After removing the roots from the bulbs, the length of 20 roots from each treatment and controls were measured using a digital paquimeter (DIGIMESS®).

***Allium cepa* statistical analysis**

Mitotic index and chromosome aberration were compared and analyzed using Kruskal–Wallis test ($p<0.05$), as suggested by Grisolia et al. (2005) e Leme e Marin-Morales (2008).

RESULTS

Antioxidant activity

Table 2 shows the results of DPPH test, FRAP and iron ion chelate to evaluate the antioxidant activity of different extracts from each spice plant evaluated in this study. It can be seen that all species have greater activity at the concentration of 1000 mg / mL. For the DPPH test the highest antioxidant activity was obtained for basil extracts (82.01 %), oregano (81.32 %) and cinnamon (77.82 %), while the other condiments presented activity below 15% (pepper and ginger = 13.49% = 12.59%). In FRAP test the highest ferric reducing power was observed for the basil extracts (321.12 uM ET / g dry extract) and oregano (315.11 uM ET / g dry extract) and the lowest activity was observed for ginger extract (9.08 uM ET / g dry extract). For the test of the iron chelating activity, all evaluated extracts showed antioxidant activity and the ginger extracts (88.57 ± 2.98), basil (94.18 ± 9.17), oregano (81.16 ± 5.80) and pepper (78.92 ± 8.34) showed no significant difference when compared to the positive control (Na_2EDTA = 90.34 ± 3.43).

There are also shown in table 2 the results of the inhibition tests of lipid peroxidation (TBARS) and kidnapping of the radical. In the TBARS test the spices cinnamon (18.52 ± 4.65), ginger (25.33 ± 5.16), basil (33.14 ± 8.94) and oregano (21.11 ± 1.67) at a concentration of 250 mg / mL showed greater inhibitory activity when compared with the positive control Trolox (47.03 ± 1.17). For the NO radical sequestration test, the species of ginger and basil showed greater activity, and there was no significant difference among the concentrations used. However for the pepper extract, it was only observed higher scavenging activity for concentrations of 250 and 500 mg mL^{-1}, which did not differ significantly from each other.

The figure 1 shows the results of antioxidant activity by oxidative hemolysis inhibition method in human erythrocytes, conducted with species of condiments with greater scavenging activity of DPPH (cinnamon, basil and oregano). It is possible to observe that the extracts presented potential to scavenge the free radical and impossible hemolytic AAPH action evaluated in concentration (100 mg mL^{-1}). In the 3rd hour the basil extract (91.82 %) did not differ significantly from the positive control (94.5 %). Otherwise the cinnamon (37.10 %) and oregano (49.68 %) extracts presented the lowest hemolytic activities and not differ among each other. In the evaluation of the 4th to the 6th hour all evaluated extracts showed oxidative hemolysis below 50% differing significantly from the positive control while cinnamon extract showed the highest antioxidant potential during evaluated period, from 37.10 % for the 3rd hour up to 12.45 % for the 6th hour.

Quantification of polyphenols and total flavonoids

The results of the quantification of polyphenols and total flavonoids are shown in Table 3. It has been found that the condiment plants presented higher levels of total polyphenols at the concentrations of 1.000 mg mL^{-1}, as the highest concentration was observed for the extract ginger (119.17 ± 6.17 mg of gallic acid equivalent / gram of dry extract) and the lowest for black pepper at concentration of 250 mg / mL (3.17 ± 0.77 mg of gallic acid equivalent / gram of dry extract).

As for determination of total flavonoids, the highest levels were observed at the concentration 1000 mg / mL, as the largest flavonoids content was observed for the ginger extract (97.56 ± 03.12 mg equivalent of quercetin / gram of dry extract) the lowest was for the black pepper extract (9.36 ± 0.16 mg equivalent of quercetin / gram of dry extract).

Table 2. Antioxidant activity of different culinary plants (cinnamon, ginger, basil, oregano and black pepper) through DPPH radical scavenging activity, ferric reducing power (FRAP), iron ion chelating power, inhibition of lipid peroxidation (TBARS), NO radical scavenging.

Treatment	Extract (mg mL^{-1})	Antioxidant Activity				
		DPPH	FRAP	Iron ion chelating	TBARS	NO
Cinnamon	250	39.46±4.23a	21.23±1.67a	23.57±3.48a	18.52±4.65a	21.06±2.03a
	500	76.23±6.17b	65.01±2.33b	30.62±2.98a	29.26±1.72b	29.02±3.65a
	1000	77.82±3.32b	142.07±4.45c	57.49±1.55c	47.22±9.82c	39.77±1.09b
Ginger	250	7.16±0.33c	3.14±0.17d	80.49±4.36b	25.33±5.16b	7.82±0.88c
	500	9.97±1.17c	5.17±0.94d	85.87±3.11b	42.77±3.09c	7.26±0.17c
	1000	12.59±1.42c	9.08±1.22d	88.57±2.98b	50.18±9.36c	8.82±0.33c
Basil	250	17.55±1.45c	87.97±3.64b	90.09±9.17b	33.14±8.94b	8.24±1.48c
	500	35.32±3.02a	163.29±3.67c	93.23±3.45b	47.96±5.70c	9.26±1.85c
	1000	82.01±4.23b	321.12±11.18e	94.18±9.17b	60.37±7.42d	12.06±1.01c
Oregano	250	49.80±2.98a	66.27±2.98b	63.89±5.11c	21.11±1.67b	33.57±1.60b
	500	75.09±3.29b	140.88±8.78c	73.95±3.12b	33.70±5.64b	45.31±6.43b
	1000	81.32±1.35b	315.11±11.33e	81.16±5.80b	59.44±2.88d	55.86±2.77d
Black pepper	250	5.19±0.98c	22.87±3.07a	58.82±4.32c	40.36±2.71c	11.68±3.74c
	500	7.29±0.97c	53.38±3.22b	61.97±2.34c	57.40± 6.24d	16.19±7.04c
	1000	13.49±1.14c	162.88±9.94c	78.92±8.34b	61.11± 1.47d	44.02±5.67b
Galic Acid	80	82.23±2.93b				
Na_2EDTA*				90.34±3.43b		
Trolox	140				47.03±1.17c	

Values are expressed as mean ±SD. Same letters within the same column indicate no significant diferences among samples by Tukey test (α≤0.05), * EDTA (1%b/v).

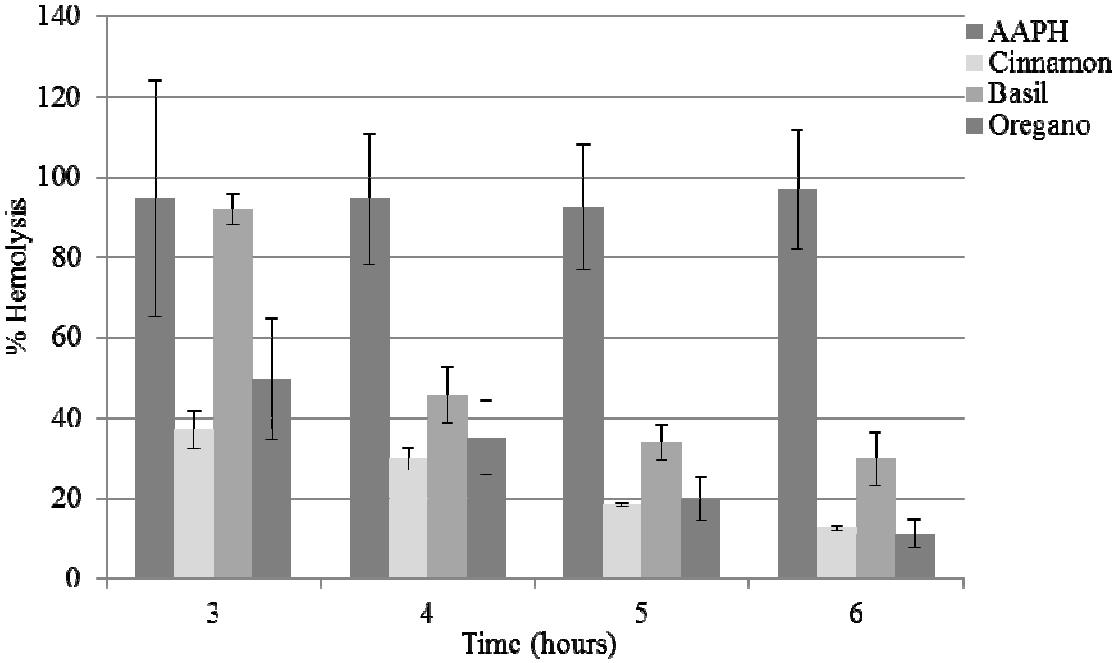

Figure 1. Antioxidant activity by hemolysis method of species of plants condiments (cinnamon, basil and oregano) and positive control (AAPH) for 6 hours incubation and measured at 540 nm in a spectrophotometer. Same letters Indicate significant diferences Among samples by Tukey test ($\alpha \leq 0.05$).

Table 3. Quantification of phenols and flavonoids totals in different condiments plant extracts (cinnamon, ginger, basil, oregano and black pepper.

Culinary Plants	Extract (mg L^{-1})	Phenol Contents[a]	Flavonoid Contents[b]
Cinnamon	250	11.78±3.19	9.08±1.10
	500	23.44±2.33	12.46±0.98
	1000	54.34±1.79	43.57±1.97
Ginger	250	45.19±3.23	40.15±1.98
	500	73.59±9.09	56.98±2.18
	1000	119.17±6.17	97.56±3.12
Basil	250	19.45±2.87	12.11±0.76
	500	33.58±1.76	32.24±1.16
	1000	64.56±2.99	53.72±3.21
Oregano	250	17.47±1.22	12.28±1.22
	500	33.28±3.02	33.04±2.76
	1000	51.52±2.19	43.72±1.44
Black pepper	250	3.17±0.77	1.16±0.09
	500	7.88±0.34	4.41±0.32
	1000	11.89±0.45	9.36±0.16

[a]Values of total phenols levels (mg of gallic acid equivalent/g of extract); [b]Total flavonoids levels in quercetin equivalent per mg/g of extract;

Bioassay *Allium Cepa*

The results of the determination the mitotic index and chromosomal aberrations are shown in Table 4. Both the analysis of the mitotic index and the chromosomal aberration of different extract concentrations of the spices did not differ from the NC. However all treatments showed significant differences compared to the PC.

Table 4. Chromosomal aberrations and mitotic index for infusion of cinnamon, oregano, basil, parsley, black pepper and ginger, in concentrations of 0.05 mg mL^{-1}, 0.1 mg mL^{-1} and 1 mg mL^{-1} negative control treated with water mineral and positive control treated with Methylmethanesulfonate (MMS) to 10 mg L^{-1}.

Treatment	Concentration (mg L^{-1})	Mitotic Index	Aberrant Anaphase	Telophase aberrant	Total of aberrant cells
CN	0,00	12.76±1.23a	3	0	3b
Cinnamon	0.05	13.62±1.31ab	3	0	3b
	0.10	12.88±1.09b	3	1	4b
	1.00	9.34±1.25c	4	1	5b
Ginger	0.05	13.90±1.76a	2	1	4b
	0.10	9.98b±1.22c	2	1	4b
	1.00	12.24±1.89ab	4	0	4b
Basil	0.05	12.78± 1.23ab	2	0	4b
	0.10	12.44±2.42ab	3	1	3b
	1.00	14.38±2.04d	3	0	3b
Oregano	0.05	13.84±2.11a	3	1	2b
	0.10	10.30±1.87b	2	1	2b
	1.00	12.14±1.12ab	4	1	5b
Black pepper	0.05	13.86±1.98a	1	1	3b
	0.10	10.40±2.98b	2	1	3b
	1.00	12.64±1.89ab	6	0	6b
CP	0.20	11.30±1.76b	38	9	47[a]

5000 cells analized; Values followed by different letters in the same row indicate significant difference ($p < 0.05$)

Mensuration of the length of *A. cepa* roots

Table 5 shows the root lengths of *A. cepa* subjected to treatments with extracts of condiment plants in concentrations of 0.05; 0.10 and 1.0 mg mL^{-1}. For the 0.05 mg mL^{-1} concentration, it was observed that all treatments showed no statistical difference when compared to PC. For the concentration of 0.10 mg mL^{-1}, in roots exposed to ginger extract were not statistically different when compared with the PC. The other treatments showed significant differences with both controls. For the concentration of 1.0 mg mL^{-1}, roots treated with oregano extract showed no significant difference when compared with NC, while cinnamon, black pepper and basil showed no significant difference when compared with PC. The length of the roots treated with ginger extract statistically differed from NC and PC.

Table 5. Length of *Allium* strain roots exposed for infusion of cinnamon, oregano, basil, parsley, black pepper and ginger at the concentration of 0.05, 0.1and 1 mg mL^{-1}. Negative control treated with mineral water and positive control treated with Methylmethanesulfonate to 10 mg L^{-1}.

Species Evaluated	Size (mm)		
	Extract (mg/mL)		
	0,05	0,10	1,00
CN	48.01±8.19a	48.01±8.19a	48.01±8.19a
Cinnamon	26.75±5.84b	24.94±6.63c	35.91±6.24b
Ginger	32.34±8.55b	29.54±9.15b	39.15±8.11c
Basil	27.14±6.66b	23.17±5.54c	33.61±9.02b
Oregano	27.21±5.52b	20.69±4.70c	50.22±5.91a
Black pepper	34.30±7.98b	26.49±7.25c	25.97±5.91b
CP	33.29±3.68b	33.29±3.68b	33.29±3.68b

20 roots analyzed with results presented in Means ± standard deviation; Equal letters column do not differ statistically, medium evaluated of Kruskal-Wallis test ($p < 0.05$).

DISCUSSION

According to Schweiggert et al. (2007) and Reinholds et al. (2015) spices and herbs of many kinds (i.e., berries, seeds, roots, fruits, bark, and leaves) have been used as substantial additives in culinary, medicinal, cosmetic and other compositions and the differences of spice quality characteristics such as color, smell, and flavor are based mainly on their biological and geographic origin. Given that the spices used in Brazilian culinary are mostly exotic species but cultivated in the geographical conditions of Brazil (Nepomuceno, 2005), this study evaluated for the first time the antioxidant and genotoxic activity of different spices (cinnamon, ginger, basil, oregano and black pepper) widely used in Brazilian culinary.

To accomplish this study it was considered the different extraction methods for each kind of spice: fresh leaves (oregano and basil) were submitted to infusion and hard parts such as seeds, barks and roots (black pepper, cinnamon and ginger) were extracted by decoction, as it is done in its use in different recipes of Brazilian culinary.

For antioxidants assays the results varied according to the evaluated condiment species. In all the assays performed in this study, the higher antioxidant activities among analyzed samples were found in basil and oregano. These results are according to Kaurinovic et al., (2011), Pitaro et al.,(2012), Zhang et al., (20S14) and Vujicic et al., (2015) who demonstrated the oregano antioxidant activity through the evaluation of different *in vitro* tests with methanolic and ethanolic extracts from the leaves of this species. The results found in this study regarding basil extracts are similar to results demonstrated by James et al., (2008), Sarfaz et al., (2011), Benedec et al., (2012), Vidovic et al., (2012) and Vardhan et al., (2013). However, these researches were realized with different extracts (aquous, ethanolic, methanolic and hexanoic) and in most cases evaluated only through DPPH test for the antioxidant activity.

Despite the basil and oregano species shown higher antioxidant activity. The other spices (cinnamon, ginger and black pepper) also obtained significant results in the antioxidant tests performed and these results are according with those found by Kim et al., (1993), Mathew and Abraham (2006a and 2006b) and Rao and Gan (2014) who demonstrated the antioxidant activity of extracts obtained from nonpolar solvents, fractions and isolated compounds from cinnamon. Stoilova et al., (2007) and Danciu et al., (2015) demonstrated the antioxidant potential of ginger ethanolic extract through the DPPH test and Chatterjee et al., (2007), Saha and Verma (2013) and Ahmad et al., (2015) demonstrated the antioxidant potential of methanolic extracts from the black pepper fruits through DPPH test and NO sequestration. Despite the number of studies showing the activity of spices aim of this study, this work shows for the first time the evaluation of the extracts obtained in a manner similar to what is possible to obtain during cooking and preparation of different Brazilian recipes, and perform a variety of *in vitro* assays to observe such activity and potential.

Considering this, we also evaluated antioxidant potential by the method of inhibiting the hemolytic power of AAPH radical on human erythrocytes with extracts of spices with the greatest antioxidant potential evaluated previously (cinnamon, basil and oregano). The results demonstrated a significant anti-hemolytic activity (antioxidant) of the extracts in the presence of AAPH radical. Although there are no reports in scientific literature regarding the anti-hemolytic activity of the spices evaluated in this study, mainly using extracts similar to those obtained in the preparation of culinary recipes. The results obtained, are according to Ferreira et al., (2007) and Pasquini-Netto et al., (2012) who demonstrated the antioxidant capacity of plant extracts by inhibiting the hemolytic activity of the AAPH radical.

Based on the antioxidant potential observed in the different spices evaluated in this study, we also searched for the total phenolic and flavonoid content of the different extracts. According to Materska and Perucka (2005) the phenolic compounds, maily flavonoids, are powerful antioxidants that can protect human body from free radicals (BORS et al., 1996; HALLIWELL, 1996; HOLLMAN et al., 1999; HARBOURNE, 2000; FERRARI et al., 2003). All evaluated spices presented levels of phenolic and flavonoid compounds while ginger was the species that presented the higher content of these compounds and the black pepper showed the lower value. The observed values are according to those found by Santiago-Ademe et al., (2015) that show the presence of phenols and flavonoids compounds in microencapsulated extracts from cinnamon infusion. Awe et al., (2013) and Nam et al., (2013) who evaluated the presence of phenols and flavonoids in ginger extracts correlating with the antioxidant activity; Uyoh et al., (2013) and Flanigan and Niemeyer (2014) who demonstrated the presence of phenolic content in basil extracts; Chrpová et al (2010) and Alezandro et al., (2011) who evaluated and found evidence regarding the presence of

phenolic compounds in oregano extracts and Ahmad et al., (2015) who demonstrated different concentrations of phenolic content in black pepper seeds.

Regarding the genotoxicity, potential evaluation it is possible to observe that the different extracts, in the analyzed concentrations, did not presented genotoxic effect both in the mitotic index analyses as in the chromosomal aberrations in meristem cells of the *A. cepa* roots test. Scientific studies has demonstrated that the condiment species evaluated in this study do not have genotoxic and/or mutagenic effect and show antimutagenic and anticarcinogenic potential evaluated by *in vivo* and *in vitro* assays as demonstrated by Vaezi et al., (2014) who observed antigenotoxic action of cinnamon infusion in human diet. Abdul et al., (2008), Beg et al., (2008) and Semwal et al., (2015) presented results of antigenotoxic, anticarcinogenic and nutraceutical activity of extracts and compounds isolated from ginger. Beric et al., (2008) who observed the antigenotoxic and cytoprotective action by comet assay from basil extract. Qari (2008), Gulluceat al., (2012) and Roa et al., (2014) who demonstrated the absence of genotoxicity and cytotoxicity of extracts and isolated compounds from oregano, while Selvediran et al. (2005) and Thiel et al. (2014) evaluated and observed the absence of genotoxic activity of black pepper seed extracts. Also, according to Bagatini et al., (2007) the *A. cepa* test is a genotoxicity assay which is sensitive and susceptible to plant compounds present in infusions and extracts, and according to Goujon et al., (2014) this assay can be used to monitoring the genotoxicity of pesticides residues in food products.

Regarding the determination of length of root A. cepa exposed to different concentrations of the spice extracts, it was observed a decrease in the root growth when compared with NC. However, this result is according with the studies performed by Fachinetto, Bagatini and Durigon (2007) and Fachinetto and Tedesco (2009) who demonstrated that the presence of phenolic compounds as tannins and flavonoids in some plant species can inhibit cell division in *A. cepa*.

CONCLUSION

The spice extracts obtained similarly to its use in wide range of Brazilian culinary recipes, can release compounds that show antioxidant activity without genotoxic effect as seem in the evaluation by *A. cepa* test.

RESUMO: As especiarias são produtos vegetais naturais, que foram utilizados não só como agentes aromatizantes e colorantes, mas também como conservantes de alimentos e medicamentos populares em todo o mundo há milhares de anos. Muitas especiarias também foram reconhecidas por ter estimulantes digestivos e ações carminativas e também potencial antimicrobiano, anti-inflamatório, antimutagênico e anticarcinogênico. O potencial antioxidante e genotóxico das espécies comumente utilizadas no Brasil foi avaliado. A atividade antioxidante foi avaliada utilizando diferentes métodos, incluindo a atividade de eliminação de radicais DPPH, poder de redução férrica (FRAP), poder quelante de íons de ferro, inibição da peroxidação lipídica (TBARS), eliminação de radicais NO e inibição da hemólise oxidativa. Além disso, a atividade antigenotóxica foi avaliada através do índice mitótico e aberração cromossômica nas raízes do *Allium cepa.* Quantificação de fenóis totais e flavonoides realizados. Os resultados mostraram que as especiarias (*Cinnamomum zeylanicum*, *Origanum vulgare*, *Piper nigrum*, *Zingiber officinale* e *Ocimum basilicum*) apresentaram atividade antioxidante avaliada por diferentes métodos e mecanismos de inativação de radicais livres e de acordo com a avaliação de genotoxicidade pelo teste *Allium cepa* as especiarias não apresentam efeitos genotóxicos.

PALAVRAS CHAVE: Antioxidant activity. Genotoxicity. *Cinnamomum zeylanicum*. *Origanum vulgare*. *Piper nigrum*. *Zingiber officinale*. *Ocimum basilicum*.

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