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Research Article
Cytotoxicity, Genotoxicity and Antioxidant Activity of Extracts from *Capsicum* spp.

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Abstract

Extracts of different genotypes of Capsicum (*C. annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L. and *C. praetermissum*) were investigated for total phenolics, flavonoids, capsaicinoids contents and antioxidant activity objectifying to determine the relations between these bioactive compounds and the antioxidant capacity and also examine its cytotoxic and genotoxic potential. *Capsicum praetermissum* showed the highest total antioxidant activity and the highest concentrations of phenolic compounds and flavonoids. For *C. annuum* and *C. frutescens* the antioxidant activity was significant and the levels of phenolic compounds and flavonoids intermediate, while *C. baccatum* demonstrated the lowest antioxidant activity independent of significant levels of these compounds. *Capsicum chinense* showed the lowest level of total phenols and flavonoids despite expressive antioxidant activity. The determination of capsaicinoid content revealed higher levels of capsaicin and dihydrocapsaicin for extracts of *C. praetermissum*, intermediate levels to *C. baccatum*, *C. chinense* and *C. frutescens*, whereas, the *C. annuum* showed the lowest levels of capsaicinoids, typical of these ornamental genotype. The extracts of *C. praetermissum* and *C. baccatum* exhibited low cytotoxic potential compared to other genotypes. The genotoxic potential was variable between analyzed genotypes, with *C. praetermissum* presenting the lowest rates. All species showed a dose-effect relationship dependent of extract concentration. The results of the present study indicated that Capsicum genotypes demonstrated a very high diversity of bioactive compounds that can be explored as agents in the control of complex biological processes related to human nutrition and health.

Key words: Peppers, chilies, capsaicinoids, micronucleus, DPPH, *Allium cepa*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The genus Capsicum (peppers and chilies) belonging to Solanaceae family is native to tropical America, comprises approximately 36 species that grow throughout the tropics and subtropics, five of which are domesticated *Capsicum annuum* L., *Capsicum baccatum* L., *Capsicum chinense* Jacquin., *Capsicum frutescens* L. and *Capsicum pubescens* Ruiz and Pavon (Eshbaugh, 2012).

The true chilies are conventionally divided into two groups, the white-flowered and the purple-flowered groups. *Capsicum praetermissum* Heiser and Smith, differenciates from the other species in that it is semidomesticated, endemic from the South East of Brazil and represents a transition between the groups of purple and white flowers. The species are predominantly diploids (2n = 24 chromosomes), present an extraordinary diversity in the fruit's morphology, color, size and flavor (from sweet to spicy) and are widely used as either vegetables or spices (Pickersgill, 1991; Csillery, 2006; Bosland and Votava, 2012; Eshbaugh, 2012; Ibiza *et al*., 2012; Wahyuni *et al*., 2011, 2013).

What draws the attention of researchers around the world for "Capsicums" is the fact that they constitute one rich source of bioactive compounds such as phenolic compounds (phenolic acids and flavonoids), carotenoids (capsanthin, β-carotene, β-cryptoxanthin, cryptoflavin), capsaicinoids (capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, nornorcapsaicin, homodihydrocapsaicin) and vitamins (C, E, A and B-complex) that exhibit functional properties as antioxidant, anticancer, anti-inflammatory, antiallergic, antimutagenic, antiviral or antibacterial (Hervert-Hernandez *et al*., 2010; Ghasemnezhad *et al*., 2011; Bae *et al*., 2012; Hallmann and Rembialkowska, 2012; Koffi-Nevry *et al*., 2012; Zhuang *et al*., 2012; Zimmer *et al*., 2012; Chen and Kang, 2013; El-Ghorab *et al*., 2013; Giuffrida *et al*., 2013; Silva *et al*., 2013; Sganzerla *et al*., 2014; Gurnani *et al*., 2015; Loizzo *et al*., 2015; Mokhtar *et al*., 2015).

More than 20 types of capsaicinoids have already been identified, having protective properties against chemical mutagens or carcinogens, analgesic and anti-inflammatory effect and have extensively been explored as agents in the control of complex processes such as diabetic neuropathy, osteoarthritis, psoriasis and obesity (Luo *et al*., 2011; Lopez *et al*., 2011; Sharma *et al*., 2013; Dong *et al*., 2014; Huang *et al*., 2014; Rollyson *et al*., 2014; Islam *et al*., 2015; Saito, 2015; Meckelmann *et al*., 2015; Sora *et al*., 2015; Srinivasan, 2015; Sung *et al*., 2015; Szolcsanyi, 2015; Yuan *et al*., 2015).

In addition to constituting a rich source of vitamins C, A (β-carotene and provitamin A), B2 (riboflavin), B3 (niacin), E, K and B6, peppers and chillies also have carotenoids (oleoresins) responsible for the pigmentation of fruits and flavonoids, which may function as antioxidant activity, anti-inflammatory, anti-allergy, hormone, anti-hemorrhagic, anti-cancer and some of these phytochemicals are widely exploited as indicators of nutritional value and quality of food (Hervert-Hernandez *et al*., 2010; Bae *et al*., 2012; Chen and Kang, 2013; Pugliese *et al*., 2014; Asnin and Park, 2015; Carvalho *et al*., 2015; Materska, 2015; Reifschneider *et al*., 2015).

The present study aimed to investigate the total phenols, flavonoids and capsaicinoids contents from extracts of *Capsicum annuum*, *Capsicum baccatum*, *Capsicum frutescens*, *Capsicum chinense* and *Capsicum praetermissum*. The antioxidant potential of each extract and the relations between each analyzed compounds and their antioxidant activity were stablished. In addition, the cytotoxicity and genotoxicity of the analyzed extracts were determined.

MATERIALS AND METHODS

Plant sample: Fourteen *Capsicum* genotypes belonging to the species *C. annuum* L., *C. baccatum* L., *C. chinense* Jacquin., *C. frutescens* L. and *C. praetermissum* Heiser and Smith, obtained from local fairs in Assis/SP, Brazil (22°39′42″S, 50°24′44″W), were used (Table 1).

Plant extracts

Preparation of plant material: The fresh fruits of 14 genotypes in mature stage without stalk or calyx were dried in an air circulating oven at 40°C for 48 h, until constant weight was obtained. Dried fruit was stored in dark plastic bottles, wrapped with aluminum foil and coated paper cover-film to maintain a low moisture and avoid contamination by microorganisms. The preparation of the extract was performed by spraying the dried fruit in a Wiley mill and the resulting powder was extracted by mechanical shaking with ethanol PA 1:10 (w/v). All experiments were performed in triplicate.

Preparation of ethanol extracts: Extracts of each genotype were prepared by mixing the power in ethanol PA at a ratio of 1:10 (w/v), then submitted to mechanical stirring for 24 h at room temperature. The vegetable residue was taken up in a low vacuum pressure. Each extraction was repeated three times. The samples were rounded up and taken to rotaevaporator for complete elimination of ethanol and submitted to dehydration. The resulting dried extracts were

Table 1: Identification of *Capsicum* spp. genotypes

Species	Polular name	Color of fruits at ripe stage
Capsicum annuum	Pimenta ornamental	Dark red
Capsicum baccatum	Pimenta lemon drop hot	Lemon green
Capsicum chinense	Pimenta bodinho	Red
Capsicum frutescens	Pimenta tabasco	Red
Capsicum praetermissum	Pimenta cumari verdadeira	Red

quantified for yield calculation (dry weight) and stored at 4°C in amber glass vials, protected from light.

Determination of antioxidant activity

Evaluation of antioxidant capacity by DPPH radical scavenging: For evaluation of antioxidant capacity against the DPPH radical, each extract was used in the *in vitro* methodology described by Blois (1958) with modifications. Each reaction mixture contained 1 mL of acetate buffer (100 mM pH 5.5), 1.25 mL of ethanol PA, 250 µL ethanolic solution of DPPH (500 µM) and 50 µL of sample. The mixture was stirred by vortexing and incubated at 25°C in the dark for 30 min. Control was measured using ethanol to replace the extracts in the reaction solution and for blank was used ethanol to replace DPPH solution. The absorbance was measured at 517 nm with UV-vis spectrophotometer (Femto-600 Plus). The test samples were done in triplicate for statistical analyzes and the absorbance values were expressed as percentage by the following equation:

$$\text{DPPH inhibition activity (\%)} = \frac{A_C - A_S}{A_C} \times 100$$

where, A_C is the absorbance of the control and A_S is the absorbance of the sample. The EC_{50} value, i.e., the concentration of sample required to cause 50% inhibition, was estimated from the plot of scavenging activity against the sample concentration, by linear regression. Same experimental procedure was used with the gallic acid positive control (80 µg mL^{-1}).

Iron ion chelating activity: The iron-chelating ability of the crude ethanolic extracts was estimated by the method described by Dinis *et al.* (1994). Six dilutions in ethanol (100, 250, 500, 1000, 1500 and 2000 µg mL^{-1}) were prepared from the dried extracts. Briefly, 0.05 mL of each dilution were added to a 2.7 mL Tris buffer (pH = 7.4). Thereafter, 0.05 mL of 2 mM $FeCl_2$ were added and vortexed for 15 sec. At 30 sec, the reaction was initiated by the addition of 5 mM ferrozine (0.2 mL), the mixture was shaken vigorously in vortex (Velp Scientifica, UE) for 10 sec. One minute after addition of $FeCl_2$, absorbance of the solution was measured at 562 nm with spectrophotometer. The ability of extracts to chelate ferrous ion was calculated relative to the control (Tris buffer, iron and ferrozine only) using the equation:

$$\text{Chelating activity (\%)} = \frac{A_C A_S}{A_C} \times 100$$

where, A_C is the absorbance of the control and A_S is the absorbance of the sample.

Scavenging activity of hydrogen peroxide (H_2O_2): A solution of hydrogen peroxide (40 mmol L^{-1}) was prepared in phosphate buffer (pH 7.4). Different concentrations (250-2500 mg mL^{-1}) of the extracts were added to the hydrogen peroxide solution (40 mmol L^{-1}, 0.6 mL). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of the extracts and standard compounds was calculated as:

$$\text{Scavenged } (H_2O_2)\ (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 was the absorbance of the control and A_1 was the absorbance the samples and in the presence of the standards, according to methodology described by Kumar *et al.* (2008).

Toxicity test in *Artemia salina*: Leach brine shrimp eggs were bought at a pet shop in Assis (São Paulo, Brazil) and incubated in artificial seawater prepared from a saline solution NaCl (34.2%), MgH_2SO_4 (1.425%), $NaHCO_3$ (4.75%), distilled water and NaOH (solution to adjust pH to 9.0) at 28±2°C and artificial light controlled according to the methodology described by Meyer *et al.* (1982) and Nunes *et al.* (2006), with modifications. After 48 h, nauplio were collected and distributed 10 individuals in each culture plate. To each plate were administered 0.1, 5 and 10 g mL^{-1} of aqueous extract and 1, 10, 50 and 100 mg mL^{-1} ethanol extracts. Number of deaths at 24 and 48 h of exposure was counted and was used to calculate the LC_{50} emploing Probit analysis, with 95% confidence according to Pagliara and Caroppo (2011). The tests were performed in triplicate according Luna *et al.* (2005).

Genotoxic activity

Mitotic index and chromosome aberration in *Allium cepa* roots: Onion bulbs (*Allium cepa* L., 2n = 16) obtained commercially in Assis, São Paulo, Brasil, 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 and Nielsen, 1993; Babatunde and Bakare, 2006). For growth of the roots was used Hoagland's solution and the bulbs were kept suspended in a 100 mL beaker 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 and 6 h light/dark) and 22±2°C in controlled chamber B.O.D. Bulbs with roots approximately 2 cm long 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 ethanolic extracts. Mineral water was used as positive control and solution of MMS (methyl methanesulfonate, Sigma-Aldrich®) at 10 mg L^{-1} as negative control as described by Carita and Marin-Morales (2008). At the end of 48 h exposure and 24 h recovery in culture solution, the roots of treated and control bulbs were cut and fixed in ethanol; glacial acetic acid (3:1, v/v), hydrolyzed in 1 N HCl at 60°C for 8 min and rinsed in distilled water. The roots were stained with acetic carmine for 10 min, the tips removed and the roots carefully smashed between slide and coverslip as described by Akinboro and Bakare (2007). Five slides 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 (Fiskesjo, 1985, 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). Mitotic index and chromosome aberration were compared and analyzed using Kruskal-Wallis test ($p<0.05$) as suggested by Leme and Marin-Morales (2009).

Total phenols and flavonoids quantification: The quantification of total phenols and flavonoids was performed with the extracts diluted in ethanol at concentrations of 100, 250, 500, 1000, 1500 and 2000 µg mL^{-1}. The Folin-Ciocalteu colorimetric method modified by Singleton and Rossi Jr. (1965) was performed to determine the total phenolic content. For each 0.5 mL of extract at different concentrations, was added 5 mL of distilled water and 0.25 mL of Folin-Ciocalteu reagent. After 3 min, 1 mL of saturated Na_2CO_3 solution at 10% was added and the mixture was stored for 1 h. The absorbance was measured at 725 nm using a UV-vis spectrophotometer (model SP220, Biospectro, Brazil). All the tests were performed in triplicate and the results expressed as Gallic Acid Equivalents (GAE) per gram of dry extract (mg of GAE g^{-1} DE).

The total flavonoid content of the crude extracts was determined using UV-Vis spectrophotometer and the samples prepared based on flavonoids complexation with $AlCl_3$ as in colorimetric method described by Zhishen *et al*. (1999). An aliquot of 250 µL of each different concentration of extracts was mixed with 1.25 mL of distilled water and 75 µL of $NaNO_2$ solution at 5% (w/v). After 6 min, 150 µL of $AlCl_3/H_2O$ solution at 10% (w/v) was added. After 5 min, 0.5 mL of NaOH 1 M solution was added and then the total volume completed by adding 2.5 mL of distilled water. The samples were shaken in a vortex mixer and the absorbance measured at 510 nm. All the tests were performed in triplicate and the results were expressed as the rutin equivalent per gram of dry extract (mg of RU g^{-1} DE).

Determination of capsaicinoid content: Capsaicinoids were quantified using HPLC by method of Collins *et al*. (1995) with modifications. Dried tissue was ground and 3 g of each sample were mixed with 30 mL of acetonitrile and kept for 4 h at 80°C with constant shaking. After the samples were cooled and filtered. A Waters HPLC instrument equipped with a Nova-Pak C_{18} reversed-phase chromatographic column of 3.9×150 mm was used in the analysis, where the mobile phase was methanol/water at a ratio of 73:27 and the flow rate was 1 mL min^{-1}. The detector was a photodiode array and the run was 7 min long.

For analysis, extracts were dissolved (10 mg mL^{-1}). Standards of capsaicin (8-methyl-n-vanillyl-6-nonenamide) and dihydrocapsaicin (8-methyl-n-vanillyl-nonanamide) were obtained from Sigma Aldrich (purity 98% for capsaicin and 90% for dihydrocapsaicin) and standard curves were prepared by serial dilutions of 100, 200, 400, 600, 800 and 1000 ppm. The results were expressed as µg g^{-1} Dry Extract (DE).

Statistical analysis: All analyzes were performed in triplicate and results presented as Means±SD. One-way analysis of variance test (ANOVA) was performed followed the Tukey test to compare any significant differences between the extracts and the controls at the 5% significance level ($p<0.05$).

RESULTS

Antioxidant activity of extracts: The results for DPPH antioxidant pattern evidenced that the greater percentage of the activity were at 10 mg mL^{-1} for all extracts (Table 2).

The species *C. praetermissum* showed the highest dose-dependent scavenging activity over the other species, reaching a level of 81.32%, very close to the positive gallic acid control (84.34%). For *Capsicum annuum*, *C. frutescens* and *C. chinense* the antioxidants activities were expressive (69.32, 54.27 and 52.17%, respectively), while *C. baccatum* demonstrated the lowest antioxidant activity (32.98%).

The analysis of behavior in relation to the antioxidant effect of iron ion chelating (Table 2), also showed higher antioxidant activity to *C. praetermissum* (79.17%), followed by *C. annuum* (49.34%), *C. chinense* (38.64%), *C. baccatum* (29.76%) and *C. frutescens* (20.18%) extracts. The results for the antioxidant activity compared to H_2O_2 sequestration (Table 2) also showed higher activity for the *C. praetermissum* extract (78.77%), superior even to gallic acid control (69.45%), followed by *C. frutescens* (34.12%), *C. chinense* (31.09%), *C. annuum* (21.98%) and *C. baccatum* (19.76%).

Table 2: Antioxidant activity of *C. annuum*, *C. baccatum, C. chinense*, *C. frutescens* and *C. praetermissum* extracts and positive control gallic acid through the DPPH test, ion iron chelation and kidnapping of H_2O_2

		Antioxidant activity		
Extracts	Concentration (mg mL^{-1})	DPPH (%)	Fe^{+2}(%)	H_2O_2 (%)
Capsicum annuum	1	21.67±2.09^a	19.23±1.29^b	8.490±0.72^a
	5	43.21±1.93^b	34.21±1.98^c	19.17±1.73^a
	10	69.32±1.09^c	49.34±2.09^c	21.98±1.77^a
Capsicum baccatum	1	17.23±2.23^a	11.23±1.89^a	7.890±0.22^a
	5	22.98±1.18^a	21.39±1.22^b	12.09±1.09^a
	10	32.98±1.22^b	29.76±1.98^b	19.54±1.67^a
Capsicum chinense	1	34.67±2.33^b	23.17±4.15^b	19.23±3.09^a
	5	45.34±3.09^b	31.57±2.98^b	23.44±1.44^a
	10	52.17±1.22^c	38.64±1.87^c	31.09±1.78^a
Capsicum frutescens	1	17.23±1.34^a	9.170±0.98^a	15.23±1.56^a
	5	32.11±1.98^b	12.17±0.82^a	22.09±1.06^a
	10	54.27±1.74^c	20.18±1.10^b	34.12±2.29^b
Capsicum praetermissum	1	42.98±2.76^b	43.49±1.76^c	34.19±2.19^b
	5	68.14±1.23^c	59.89±2.11^c	47.18±1.29^b
	10	81.32±2.31^d	79.17±2.31^d	78.77±2.78^c
Gallic acid	0.1	84.34±1.59^d	86.94±1.74^d	69.45±3.64^c

Values with the same letter in the column do not differ significantly (Tukey: $\alpha \leq 0.05$)

Table 3: Toxicity test for *Artemia salina* to *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. praetermissum* and extracts percentage of death from observation time, CL_{50} and confidence level-95%

		Mortality (% h^{-1})			
Treatments	Concentration (µg mL^{-1})	24	48	Values CL_{50}* (µg mL^{-1})	Confidence level 95% (µg mL^{-1})**
Capsicum annuum	50	63	72	78.14	71.23-99.17
	100	71	79		
	500	83	91		
	1000	92	99		
Capsicum baccatum	50	55	67	61.17	43.21-89.23
	100	59	68		
	500	65	73		
	1000	69	81		
Capsicum chinense	50	81	97	80.76	79.93-100
	100	93	100		
	500	98	100		
	1000	100	100		
Capsicum frutescens	50	77	87	77.98	66.94-100
	100	82	98		
	500	92	100		
	1000	97	100		
Capsicum praetermissum	50	36	49	63.22	57.31-93.28
	100	51	63		
	500	68	79		
	1000	73	91		

*CL_{50} calculated after 48 h of exposure, **Pagliara and Caroppo (2011)

Table 4: Mitotic index (IM), chromosomal abnormalities index (IAC), mutagenicity Index (IM^T), chromosomal aberrations and micronucleus in onion root cells exposed to extract of *C. annuum*, *C. baccatum*, *C. frutescens*, *C. chinense* and *C. praetermissum*, Negative Control (NC) treated with mineral water and Positive Control (CP) treated with metilmetanosulfonato (MMS)

Treatments	Concentration (μg mL^{-1})	IM[a]	IAC[b]	IMt[c]	Metaphase aberrant	Anaphase aberrant	Telophase aberrant	Micronucleus
NC	0	13.20±1.27[a]	0.97±0.25[a]	0.62±0.15[a]	2.11±1.23[a]	1.44±0.79[a]	0.32±0.11[a]	0.87±0.15[a]
Capsicum annuum	10	11.44±1.02[a]	3.76±0.47[b]	2.12±0.19[c]	3.41±0.95[b]	3.50±1.24[b]	1.23±0.30[b]	1.82±0.84[b]
	50	16.52±1.15[b]	6.24±0.10[d]	3.70±0.65[c]	3.82±0.94[b]	3.69±1.05[b]	1.98±0.18[b]	2.25±0.48[b]
	100	22.04±1.67[c]	8.54±0.63[e]	5.18±0.58[c]	2.96±0.37[b]	4.09±1.07[b]	2.17±0.11[b]	2.92±0.39[b]
Capsicum baccatum	10	17.15±0.40[b]	7.12±0.77[e]	3.60±0.52[c]	3.20±0.69[b]	3.18±0.39[b]	1.94±0.30[b]	1.60±0.58[b]
	50	19.32±1.46[b]	7.76±1.20[e]	4.36±0.69[c]	3.24±0.44[b]	4.03±0.71[b]	2.09±0.39[b]	2.12±0.77[b]
	100	24.84±1.35[c]	9.66±0.91[e]	5.30±0.32[c]	4.30±0.16[b]	4.82±0.76[b]	2.41±0.28[b]	2.80±0.58[b]
Capsicum chinense	10	12.34±1.07[a]	6.84±0.60[d]	3.62±0.38[c]	3.69±0.97[b]	3.32±0.11[b]	2.60±0.36[b]	2.62±0.14[b]
	50	14.92±0.94[a]	8.16±0.93[e]	4.94±0.52[c]	4.30±0.32[b]	3.70±0.70[b]	2.78±0.58[b]	2.94±0.40[b]
	100	19.00±0.91[b]	9.10±0.37[e]	5.76±0.53[c]	4.82±0.79[b]	5.82±1.32[b]	3.04±0.07[b]	3.08±0.70[b]
Capsicum frutescens	10	13.60±2.01[a]	2.32±0.23[b]	0.68±0.19[a]	2.21±1.51[a]	3.05±0.81[b]	0.18±0.10[a]	1.72±0.14[b]
	50	13.40±1.50[a]	3.14±0.46[b]	0.72±0.16[a]	3.16±2.07[b]	3.82±6.44[b]	1.44±0.07[b]	1.92±0.54[b]
	100	10.50±1.13[a]	4.10±0.58[c]	0.94±0.18[b]	4.31±3.01[b]	4.07±9.11[b]	1.68±0.09[b]	2.19±0.44[b]
Capsicum praetermissum	10	12.00±1.14[a]	2.34±0.47[b]	0.88±0.06[a]	1.96±0.88[a]	1.07±0.58[a]	0.40±0.07[a]	0.23±0.03[a]
	50	14.20±0.22[a]	2.62±0.46[b]	0.40±0.05[a]	2.08±0.48[a]	2.02±0.30[a]	0.51±0.02[a]	0.52±0.09[a]
	100	14.40±0.46[a]	2.98±0.24[b]	0.24±0.03[a]	2.49±0.36[a]	2.22±0.92[a]	0.72±0.02[a]	0.90±0.22[a]
PC		8.60±0.72[d]	12.18±0.59[f]	8.22±0.31[d]	7.56±5.30[c]	7.64±1.67[c]	6.26±1.34[c]	7.84±3.29[c]

[a]Mitotic index (total No. of dividing cells/total cells analysed×100), [b]Index of chromosomal changes (total No. of altered cells/total cells analyzed×100) e, [c]Index of equal letters in column do not differ statistically, means evaluated with Kruskal-Wallis test ($p<0.05$)

Toxicity test in *Artemia salina*: The cytotoxic *Artemia salina* test for different concentrations of the extracts of *Capsicum annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. praetermissum* exhibit variable levels of mortality after 24 and 48 h of observation and LC_{50} between 61.17 and 80.76 μg mL^{-1} (Table 3). The extracts of *C. praetermissum* and *C. baccatum* exhibited the lowest mortality rates after 24 and 48 h (36-91 and 55-81%, respectively), with LC_{50} between 61.17 and 63.22 mg L^{-1}, showing the low cytotoxic potential of these species. For extracts of *C. frutescens*, *C. annuum* and *C. chinense*, the *A. salina* mortality rates were higher, ranging from 63-100%, with LC_{50} 77.98, 78.14 and 80.76 mg L^{-1}, respectively. In all extracts, however, it was found that mortality was proportional to the increase in concentration, which corresponds to the linearity of the dose-effect relationship of each statement, especially after 48 h of exposure.

Genotoxicity: The Mitotic Index (MI) of root meristem cells of *Allium cepa* treated with the ethanolic extracts showed variation among the analyzed species (Table 4). The values of the MI of *C. frutescens* did not differ significantly from mineral water negative control (13.2) even in relation to the extract concentration (13.6, 13.4 and 10.50) but were higher than the positive control MMS (8.60). In *C. annuum* the MI was variable in relation to the concentration of the extract. The MI observed in the lowest concentration of the extract (11.44) was not significantly different from the negative control (13.2). However, the MI in the concentrations of 50 and 100 μg mL^{-1} were statistically different (16.52 and 22.04, respectively) in relation to negative and positive controls.

In *C. praetermissum*, no significant differences were observed between the MI in the different concentrations of extracts (12.0, 14.2 and 14.4) and the negative control (13.2) but the same MI were statistically different from the MMS positive control (8.6). *Capsicum baccatum* showed the highest MI values and all concentrations of the extracts (17.15, 19.32 and 24.84) differed significantly from the positive (13.2) and negative controls (8.60). The MI of *C. chinense* observed for concentrations of 10 and 50 μg mL^{-1} (12.34 and 14.92, respectively) did not differ statistically from the negative control (13.2), despite the significant difference from the positive control (8.60). However, the highest concentration of extract (100 μg mL^{-1}) showed higher MI (19.0) and statistically differed from the positive and negative controls.

The analysis of the rate of chromosomal changes (IAC) showed that all evaluated *Capsicum* species have a dose-effect relationship dependent of extract concentration and differed significantly from negative controls (0.97 and 12.18, respectively). Comparatively, *C. praetermissum* showed the lowest IAC (2.98), followed by *C. frutescens* (4.10), while *C. annuum* (8.54), *C. chinense* (9.10) and *C. baccatum* (9.66) showed the highest IAC. The mutagenicity index (IMT) also showed a dose-dependent behavior of the extract concentration for the species in question, except for *C. praetermissum*, whose change has been reversed since the

Table 5: Determination of total content of polyphenols, flavonoids, capsaicin and dihydrocapsaicin of total extracts of *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. praetermissum*

Extracts (10000 μg mL^{-1})	Total polyphenols (mg GAE g^{-1} DE)	Total flavonoids (mg RU g^{-1} DE)	Capsaicin (μg g^{-1} DE)	Dihydrocapsaicin (μg g^{-1} DE)
Capsicum annuum	341.78±45.44	123.56±31.49	893.17±78.93	678.21±87.21
Capsicum baccatum	156.76±15.71	98.76±40.25	2697.84±110.85	1194.09±98.75
Capsicum chinense	107.98±17.14	67.84±11.09	1987.65±154.18	1542.97±122.34
Capsicum frutescens	124.47±19.41	94.96±19.05	1117.32±94.98	987.02±57.43
Capsicum praetermissum	498.08±49.90	388.49±34.56	5119.07±159.34	3132.98±79.04

Data are expressed as Mean±SD (Standard Deviation)

higher concentration extract the IMT was the lowest (0.24), even compared to other species. *Capsicum frutescens* showed a low IMT (0.94) but statistically different from the negative control (0.62), while *C. annuum*, *C. baccatum* and *C. chinense* were not statistically different (5.18, 5.30 and 5.76, respectively) but differed the negative control (0.62) and were below the positive control (8.22).

In the analysis of chromosomal aberrations and micronuclei (Table 4) significant variations were detected for *C. frutescens*, *C. annuum*, *C. baccatum* and *C. chinense*. These variations were higher only compared to negative controls, with dose-dependent rates of concentration of the extract and significantly reduced when compared to the positive controls. For the species *C. praetermissum* all indexes related to chromosomal aberrations and micronuclei did not differ significantly compared to the negative control, regardless the concentration of the extract evaluated.

Total phenols and flavonoids quantification: Results of the quantification of total phenols (Table 5) were higher for *C. praetermissum* and *C. annuum* (498.08 and 341.78 mg GAE g^{-1} DE, respectively), followed by *C. baccatum* and *C. frutescens* (156.76 and 124.47 mg GAE g^{-1} DE, respectively), whereas, *C. chinense* showed the lowest level of total phenols (107.08 mg GAE g^{-1} DE). Similar pattern was observed in the results of quantification of total flavonoids, where, to *C. praetermissum* the index was higher (388.49 mg RU g^{-1} DE), followed by *C. annuum*, *C. frutescens* and *C. baccatum* with intermediate levels (123.56, 98.76 and 94.96 mg RU g^{-1} DE, respectively), whereas, *C. chinense* showed the lowest level of total flavonoids (67.84 mg RU g^{-1} DE).

Determination of capsaicinoid content: The determination of capsaicinoid content (Table 5) showed higher levels of capsaicin and dihydrocapsaicin for *C. praetermissum* extracts (5119.07 and 3132.98 μg g^{-1}, respectively), whereas, intermediate levels were obtained from extracts of *C. baccatum* (2697.84 and 1194.09 μg g^{-1}), *C. chinense* (1987.65 and 1542.97 μg g^{-1}) and *C. frutescens* (1117.32 and 987.02 μg g^{-1}). The *C. annuum* extracts showed the lowest levels of capsaicin (893.17 μg g^{-1}) and dihydrocapsaicin (678.21 μg g^{-1}).

DISCUSSION

Traditionally, several species and varieties of Capsicum have been explored for their functional properties as an antioxidant, anti-inflammatory, anti-allergenic, anti-bacterial, antimutagenic. It is likely that such potential is directly related to the occurrence and expression of bioactive compounds in these plants, such as phenolic compounds, carotenoids, capsaicinoids and vitamins (Hervert-Hernandez *et al*., 2010; Ghasemnezhad *et al*., 2011; Bae *et al*., 2012; El-Ghorab *et al*., 2013; Koffi-Nevry *et al*., 2012; Zhuang *et al*., 2012; Chen and Kang, 2013; Silva *et al*., 2013; Loizzo *et al*., 2015; Mokhtar *et al*., 2015).

In general, scientific development in the area has focused the analysis of "grown capsicums" as *C. annuum*, *C. baccatum*, *C. chinense* and *C. frutescens* (Hallmann and Rembialkowska, 2012; Zimmer *et al*., 2012; Giuffrida *et al*., 2013; Sganzerla *et al*., 2014; Gurnani *et al*., 2015). Little has been achieved in understanding wild and semi-cultivated *Capsicum* species, such as *C. praetermissum* (Kobata *et al*., 2008; Tanaka *et al*., 2009; Da Costa *et al*., 2009; Loizzo *et al*., 2015).

This study aimed to evaluate the profile of phenols, flavonoids and capsaicinoids in genotypes of the cultivated species *C. annuum*, *C. baccatum*, *C. chinense* and *C. frutescens* and semi-cultivated species *C. praetermissum* and establish relations between these compounds and antioxidant activity, cytotoxic and genotoxic, search for information to clarify the functional potential of horticultural products so appreciated in the world.

The evaluation of total phenols content showed a lower amount of polyphenols for *C. chinense*, intermediate values for *C. baccatum* and *C. frutescens* and expressive values to *C. annuum* and *C. praetermissum* and this trend was also observed for the total flavonoids content. Research by Wahyuni *et al*. (2011, 2013), Loizzo *et al*. (2015) and Sora *et al*. (2015) showed differential composition and content of total

phenols and other bioactive compounds in extracts from fruits at different stages of maturation of cultivated and semi-cultivated types of Capsicum. In the present study, only ripe fruit were evaluated and their phenols content, flavonoids and capsaicinoids as well as antioxidant activity. Results here obtained were superior to other species such as those reported by Da Costa *et al*. (2009), Hervert-Hernandez *et al*. (2010), Zimmer *et al*. (2012), Zhang *et al*. (2015), Dong *et al*. (2014) and Carvalho *et al*. (2015).

For contents of capsaicin and dihydrocapsaicin found, *C. frutescens* and *C. annuum* showed less significant values compared to *C. baccatum* and *C. chinense* and such content are in accordance with those found by Carvalho *et al*. (2015) and Neitzke *et al*. (2015), who reported significant variation in capsaicin concentration in *C. baccatum* and *C. chinense*. The diversity between the results reported in the present study and the literature may be related to extensive variability found among the different types of peppers and chilies, either because of genetic diversity itself among the accessions, the occurrence of numerous botanical varieties within the different species, the geographical origin of accessions, the variation in maturity levels of plants or because of the methods used for extraction and analysis of bioactive compounds.

Capsicum praetermissum showed very high levels of capsaicinoids as well as total phenols, flavonoids and antioxidant activity, corroborating Da Costa *et al*. (2009), that evaluated the antioxidant activity of crude extract and hexane, chloroform and ethyl acetate fractions of malagueta peppers (*C. frutescens*), cambuci (*C. baccatum* var. *pendulum*), cumari (*C. baccatum* var. *praetermissum*) and peppers magali (*C. annuum* var. *annuum*) by β-carotene/linoleic acid system, DPPH test and the concentrations of capsaicinoids and phenolics. Even using more polar solvents the authors demonstrated higher concentration of phenolic and total capsaicinoids in hexane and ethyl acetate fractions of *C. praetermissum* as well as improved antioxidant activity by DPPH method for this species and to *C. baccatum* indicated and the use of these peppers as natural antioxidants in food.

This significant result for *C. praetermissum* may be related to the fact that this species is semi-grown and have a stronger secondary metabolism as a defense mechanism against herbivory. This finding opens up prospects for the use of this species as a source of bioactive compounds, which can be exploited in the pharmaceutical area since these compounds have antioxidant potential, for example, to reduce the concentration of cholesterol in the plasma and exhibit vasodilating activity preventing cardiovascular disease as indicated by Huang *et al*. (2014).

The highest free radical scavenging ability of the ripe fruit extracts by method DPPH was observed at a concentration of 10 mg mL^{-1}. The lowest antioxidant activity was observed for *C. baccatum* following intermediate levels to *C. annuum*, *C. frutescens* and *C. chinense* and a higher level in *C. praetermissum*, including very close to gallic acid control. Considering that free radicals and reactive oxygen species are associated with many pathological conditions such as inflammation, metabolic disorders, cell ageing and carcinogenesis, the potential use of these species can contribute to the control of these disorders by intercepting or preventing the oxidative damage caused by such radicals.

Studies conducted by Loizzo *et al*. (2015) evidenced low polyphenols and capsaicinoids content for *C. baccatum*, reflecting lower antioxidant activity and high levels of these compounds in cultivars of *C. annuum* indicating higher antioxidant activity. However, the authors suggest that it seems there is no direct correlation between antioxidant status and the amount of phytochemicals, such peppers and the individual classes of these evaluated compounds are weakly associated with the antioxidant parameters, even in tests that share the same mechanism of action. In contrast, Carvalho *et al*. (2015) related that antioxidant property of peppers could presumably be attributed to the content of phenolic compounds since there was a significant correlation of the phenolic compounds content with both methods used to evaluate the antioxidant activity.

The antioxidant activity determined regarding effect of chelating iron ion and H_2O_2 sequestration was concentration-dependent in all analyzed genotypes and higher in *C. praetermissum* extract compared to the others, which increases the potential use of this species as chemopreventive able to delaying or preventing catalyzed oxidative damage by free radicals, such as reactive oxygen species related to cellular aging process and activation of carcinogenesis.

The evaluation of the cytotoxic potential of extracts of different genotypes against *Artemia salina* test found variable levels of mortality, proportional to the increase of the concentrations of the extracts. *C. baccatum* and *C. praetermissum* showed lower cytotoxic potential compared to other cultivated species. Regarding the genotoxicity of the extracts, the indices evaluated (mitotic index, rate of chromosomal changes, mutagenicity index and chromosomal aberrations and micronuclei) were adequate to show that *C. frutescens* and *C. praetermissum* have no genotoxicity, while and *C. annuum* showed potential genotoxic only at higher concentrations of the extracts. To

C. baccatum, the potential genotoxic was more expressive even at lower concentrations of the extracts, whereas, *C. chinense* this behavior occurred only at the highest concentration. In all cases, where the genotoxicity was found, it was shown to have dose-dependent relationship effect of extract concentration, higher compared to controls negative and significantly reduced when compared to the positive controls.

For different types of extracts from various species of Capsicum, medicinal properties are attributed, for example, antimicrobial activity, pesticidal and healing as indicated by Gurnani *et al*. (2015). However, it is still incipient the report number on the toxicological analysis of these species. According to Leme and Marin-Morales (2009), the *Allium cepa* test has wide potential to assess chromosomal abnormalities and mitotic cycle disorders and consequently the genotoxicity and cytotoxicity of several chemical compounds. The evaluation of the cytotoxic and genotoxic effects of *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. praetermissum* extracts through the test with *Allium cepa* showed a differentiated behavior among genotypes. The effects were dependent on the concentration of the extract and all the tested extracts were observed to have mitodepressive effects on cell division and induced mitotic spindle disturbance in *A. cepa*, suggesting an inhibitory, mitodepressive and turbagenic activities of the extracts, corroborating results obtained by Akinboro and Bakare (2007) in a study of medicinal plants.

The determination of mitotic index in root meristematic cells of *A. cepa* added significance in assessing the potential genotoxic of the different extracts and their fractions (De Pinho *et al*., 2010). El Hamss *et al*. (2003) report that varieties of *C. annuum* may contain potentially chemoprotectors compounds such as phenols and capsaicinoids that interact in synergistic and additive mode giving these species antimutagenic activity.

CONCLUSION

This study showed that there is extensive variability in the content of total phenols, flavonoids and capsaicinoids among the analyzed species of Capsicum and that there is direct relationship between their content and the antioxidants activities demonstrated by conventional methods such as test DPPH and effect of chelating iron ion and H_2O_2. The species *C. praetermissum* showed very significant content of bioactive compounds, more significant antioxidant activity and levels of toxicity and genotoxicity reduced compared to other analyzed genotypes. This finding opens up prospects for the use these species as a source of bioactive compounds, which can be exploited how chemoprotectors.

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