BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902017000216098

Article

Preliminary *in vitro* assessment of the potential toxicity and antioxidant activity of *Ceiba speciosa* (A. St.-Hill) Ravenna (Paineira)

Camila Krüger Cardoso Malheiros[1,*], Joyce Sayonara Barbosa Silva[1], Tânia Cristiane Hofmann[1], Thiane Martins Messina[1], Vanusa Manfredini[1], Jacqueline da Costa Escobar Piccoli[1], Débora Faoro[2], Luís Flávio Souza Oliveira[1], Michel Mansur Machado[1], Fabiane Moreira Farias[1]

[1]Faculty of Pharmacy, Federal University of Pampa, Uruguaiana, RS, Brazil, [2] Federal University of Santa Maria, Cachoeira do Sul, RS, Brazil

The bark tea of *Ceiba speciosa*, a tropical tree of the Malvaceae family, is used in the Northwestern Region of Rio Grande do Sul state, Brazil, to reduce blood cholesterol levels. However, there are no scientific data on the efficacy and safety of this plant. The aim of the present study was to evaluate the *in vitro* antioxidant and toxic potential of bark extracts of *C. speciosa*. We performed a preliminary phytochemical analysis by high-performance liquid chromatography-diode array detection (HPLC-DAD) and evaluated the oxidative damage to proteins and lipids, the radical scavenging effect, and genotoxicity of the lyophilized aqueous extract (LAECs) and the precipitate obtained from the raw ethanol extract (Cs1). The phytochemical profile demonstrated the presence of phenolic and flavonoid compounds. The LAECs and Cs1 prevented damage to lipids and proteins at concentrations of 50 and 10 µg/mL. They also showed a scavenging effect on 2,2-diphenyl-1-pricril-hydrazyl (DPPH) radicals in a concentration-dependent manner. Furthermore, no genotoxic effect was observed at concentrations of 10, 5 and 2 µg/mL in the Comet assay. The present study is the first evaluation regarding the characterization of *C. speciosa* and its safety, and the results demonstrate its antioxidant potential and suggest that its therapeutic use may be relatively safe.

Uniterms: *Ceiba speciose/*phytochemistry. *Ceiba speciose/*antioxidant activity. Phenolic content. Comet assay. DPPH scavenging capacity. Natural medicine. Safety.

INTRODUCTION

Given the importance of medicinal plants as sources of new drugs, ethnopharmacology is an important tool for the discovery of species with potential biological activity (Carlini *et al.,* 2006), even in the absence of scientific information on the safety and efficacy of these species. This branch of science studies traditional knowledge and use of plants as alternatives to essential resources for survival, such as food and medicine (Balick, Cox, 1996). Studies of medicinal plants are also of fundamental importance since many of them have a strictly local use and may offer an alternative treatment for various diseases. The popularization of medicinal plants has made them easier to acquire; however, there remains a lack of adequate information about the properties of the plants used and their possible interactions with prescribed drugs, as well as problems in botanical identification, and ignorance in terms of possible side effects (Mashwani *et al.*, 2015; Medeiros, Ladio, Albuquerque, 2015). Technological advances in healthcare as a whole, and, in particular the wide range of treatments and drugs available, have not meant the end of the use of medicinal plants as a therapeutic alternative for a large part of the population. In fact, in recent decades, as well as the ever-increasing use of medicinal plants, approximately 30% of all lead compounds evaluated as therapeutic agents have been derived from natural products (Calixto, 2005). Brazil is no exception in this context; therefore, in 2007, the Brazilian Ministry of Health published The National

***Correspondence:** C. K. C. Malheiros. Faculdade de Farmácia. Universidade Federal do Pampa. BR 472, Km 592, Caixa Postal 118, 97508-000 - Uruguaiana - RS, Brazil. E-mail: camila.kcm@gmail.com

Policy on Medicinal Plants and Herbal Medicines (Brasil, 2007), aimed at the implementation of the therapeutic use of medicinal plants in the Brazilian Unified Health System (SUS). The main aims of this policy were to encourage research focused on ensuring the proper and safe use of these species. In addition, the National List of Medicinal Plants of Interest to Brazilian Public Health System (RENISUS), containing 71 species for therapeutic purposes (Brasil, 2009), was created in order to guide studies and research that support the development of a list of medicinal plants and herbal medicines to be made available for use by the general population.

One of the most significant contributors to toxicity and the development of various diseases are free radicals; however, they are fundamental to biochemical processes and represent an essential part of aerobic life and metabolism. Under normal conditions, reactive oxygen species (ROS) are an essential part of such processes, and the most common ROS include the superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), peroxyl radicals ($ROO^{\bullet}$), and reactive hydroxyl radical ($HO^{\bullet}$) (Benhammou, Bekkara, Panovska, 2009; Gaur *et al*., 2009). The imbalance between free radical production and endogenous detoxification systems (predominantly non-enzymatic and enzymatic antioxidants) can lead to oxidative stress. Damage occurs mainly through the interaction of these radical species and macromolecules such as lipids, proteins, and deoxyribonucleic acid (DNA) (Benhammou, Bekkara, Panovska, 2009). The interaction of free radicals with DNA can lead to mutations and may therefore promote carcinogenesis, and accentuate the processes involved in the development of atherosclerosis, arthritis, and ischemia. ROS detoxification systems include enzymatic and non-enzymatic antioxidants. Enzymatic systems include superoxide dismutase, catalase, glutathione peroxidase, and other enzymes that act specifically against ROS (El-Shenawy, Mohammadden, Al-Fahmie, 2012). Non-enzymatic systems, either endogenous in origin or obtained through the diet, include glutathione, vitamin C, indoles, catechols and polyphenols, bioflavonoids, vitamin E, and carotenoids (Kharrazi *et al*., 2008). The demand for antioxidants means that plant materials are a significant resource in the fight against diseases associated with oxidative stress.

Despite their popular medicinal use for many different purposes, especially in South America, there have been few pharmacological studies of species of the genus *Ceiba,* which is not listed by RENISUS (Said, Nahla, Ehsan*,* 2013). Popularly known as silk tree, kapok and "barriguda", trees of this genus belong to the family *Malvaceae* and are mainly found in rainforest areas. *Ceiba speciosa* (A. St. Hill) is a tropical tree species with a wide geographical distribution that is usually found in the mesophytic semideciduous forests of Paraguay, Argentina and Brazil. In Brazil, they occur abundantly in the states of Rio de Janeiro and Minas Gerais (Beleski-Carneiro, Suqui, Reicher*,* 2002). In the state of Rio Grande do Sul, this plant is found mainly in the forest region of Alto Uruguai. This species is also a popular ornamental tree in parks and gardens because of its undemanding soil requirements, achieving satisfactory growth even in dry and sandy soils with poor chemical fertility (Veloso *et al.*, 1997; Cappelatti, Schmitt, 2009). The scientific literature describes various popular medicinal uses of several species of the genus *Ceiba* (Cartaxo, Souza, Albuquerque, 2010; Scarpa, 2004; Ladeji, Omekarah, Solomon, 2003). There are reports of the popular use of this plant in the northwest region of the state of Rio Grande do Sul for the reduction of serum cholesterol, triglyceride and glucose levels. The present study aimed to evaluate the *in vitro* antioxidant potential and toxicity of *C. speciosa*, contributing to our knowledge about this poorly studied plant; and to increase ethnopharmacological knowledge by collecting some phytochemical, pharmacological, and toxicological data about the species.

MATERIAL AND METHODS

Chemicals

All chemicals used were of analytical grade. High-performance liquid chromatography (HPLC) solvents were purchased from Merck (Darmstadt, Germany). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Plant material

The plant material was collected in May 2012 in the municipality of Santo Antônio das Missões, Rio Grande do Sul, Brazil. The species was identified and a voucher specimen (RSPF 12367) was deposited in the Herbarium of the University of Passo Fundo, Passo Fundo, Rio Grande do Sul, Brazil.

Preparation of aqueous extract

The aqueous extract was prepared by decoction of the powder from the dry trunk bark of *C. speciosa.* The obtained powder was boiled in distilled water for 30 minutes with periodic stirring. This procedure was adopted to simulate the popular use of this plant as a tea.

The extract was filtered and the filtrate was lyophilized resulting in the lyophilized aqueous extract of *C. speciosa* (LAECs).

Preparation of ethanolic extract

The raw ethanolic extract of *C. speciosa* was obtained by maceration until exhaustion at room temperature after which the solvent was removed under reduced pressure at 45 ºC using a rotary evaporator. During this process, a large amount of brown precipitate formed (Cs1). Little material remained in the ethanol extract after Cs1 separation, and it was thus discarded. Like the aqueous extract, Cs1 was rich in phenolic compounds and was therefore used for experiments. Further investigations on the complete ethanolic extract are being carried out.

Phytochemical analysis

Determination of total polyphenolic content

Measurement of total polyphenols was performed in accordance with the method established by Chandra and Mejia (2004) using a final concentration of 0.150 mg/mL. The standard curve was prepared using gallic acid. All analyses were performed in triplicate.

Determination of total flavonoid content

The total flavonoid content of LAECs and Cs1 was determined in accordance with Zhishen, Mengcheng and Jianming (1999), with a starting concentration of 1 mg/mL. The standard curve was prepared using quercetin, and all analyses were performed in triplicate.

Analysis by high-performance liquid chromatography with diode array detection (HPLC / DAD)

Lyophilized aqueous extract of *C. speciosa* (LAECs) and Cs1 were submitted to preliminary chromatographic analysis to determine their chromatographic profiles and the spectra of their main compounds in the ultraviolet region with emphasis on the quantification of flavonoids and phenolic compounds (quercetin, rutin, caffeic acid, gallic acid, chlorogenic acid, rosmarinic acid and kaempferol). High performance liquid chromatography (HPLC) of the samples was performed with an HPLC system (Shimadzu, Kyoto, Japan) consisting of a prominence auto sampler (SIL-20A), equipped with Shimadzu LC-20 AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A UV-VIS-DAD (diode-detector), and Software LC solution 1.22 SP1, according to the method described by Boligon *et al*. (2009) with minor modifications. The analyses were carried out with a C18 analytical column (5 µm; 4.6 x 250 mm) and a gradient combining solvent A (1% formic acid in water) and B (acetonitrile) with a flow rate of 0.7 ml/min (Table I). The UV absorption spectra of standards as well as the samples were recorded in the range of 230-400 nm. Table II shows the detection wavelengths of phenolic compounds analyzed.

TABLE I - Gradient elution used for the evaluation of lyophilized aqueous extract (LAECs) and precipitate obtained from the raw ethanol extract (Cs1). Solvent A (1% formic acid in water). Solvent B (acetonitrile)

Time (min)	Flow (mL/min)	B (%)	A (%)
10	0.7	87	13
20	0.7	80	20
30	0.7	70	30
40	0.7	50	50
50	0.7	40	60
60	0.7	30	70
70	0.7	80	20
80	0.7	90	10

TABLE II - Wavelengths used for the detection of phenolic compounds by High-Performance Liquid Chromatography with Diode Array Detection (HPLC/DAD)

Phenolic Compound	Wavelengths (nm)
Gallic Acid	254
Caffeic, Ellagic, and Chlorogenic Acids	325
Quercetin, Rutin, and Kaempferol	365

In vitro toxicological and antioxidant evaluation

Leukocyte and plasma samples

The experimental protocols used for the cytotoxicity and genotoxicity analyses, including venous blood collection, were approved by the Research Ethics Committee of the Federal University of Pampa (Universidade Federal do Pampa [UNIPAMPA]), under registration number 27045614.0.0000.5323.

The venous blood was collected by venipuncture after 12 hours' overnight fasting using top purple Vacutainer® (BD diagnostics, Plymouth, UK) tubes with heparin from a single healthy non-medication-using donor aged over 18 years old. The blood sample was centrifuged at 3000 rpm for 10 minutes and the white

pellet (leukocytes) was transferred to another test tube with phosphate-buffered saline (PBS; pH 7.4). The number of leukocytes was standardized (8×10^3 cells/mL) for measurements of cell viability and oxidative damage of DNA (Pereira *et al*., 2015).

Cell viability test

Cell viability in human leukocytes was assessed by the loss of membrane integrity using the Trypan Blue method (Burow *et al*., 1998) to determine the concentrations of LAECs and Cs1 to be used in *in vitro* tests. Stock solutions of LAECs and Cs1 (2 µg/mL) were prepared in PBS. From these, aliquots were transferred to Eppendorf tubes and diluted in a suspension of leukocytes to achieve concentrations of 1000, 750, 500, 250, 100, 50, 10, 5 and 2 µg/mL. Phosphate-buffered saline with H_2O_2 was used as the positive control. The negative control consisted of a PBS and leukocyte suspension. The concentrations chosen for this study showed cell viability above 85%. The analysis was performed in triplicate.

Evaluation of oxidative damage and antioxidant potential

Radical-scavenging capacity – DPPH assay

Evaluation of *in vitro* antioxidant capacity was performed by measuring the abilities of LAECs and Cs1 to quench the 2,2-diphenyl-1-pricril-hydrazyl (DPPH) radical, according to the method of Sharma and Bhat (2009). The analysis was performed in triplicate.

Lipid peroxidation test

Assessment of lipid damage was performed by quantification of thiobarbituric acid reactive species (TBARS) (Ohkawa, Ohishi, Yagi, 1979). Initially, LAECs and Cs1 solutions were prepared at the previously chosen concentrations. Two hundred microliters of plasma were mixed with 100 µL H_2O_2 100 µmol/L (1:10 dilution) and 200 µL LAECs or Cs1 solutions. For the positive control 200 µL H_2O_2 was used in place of the LAECs and Cs1 solutions. In the negative control, they were replaced by saline solution. The analysis was performed in triplicate.

Determination of protein carbonyl groups

Determination of protein carbonyl groups was performed according to the method of Morabito *et al*. (2004). Initially, LAECs and Cs1 solutions were prepared at the previously chosen concentrations. To perform the test, 200 µL plasma was added to an Eppendorf tube and mixed with 100 µL H_2O_2 solution 100 µmol/L (1:10 dilution), and 200 µL LAECs or Cs1 solutions. As a positive control, 200 µL H_2O_2 was used in place of the LAECs and Cs1 solutions. In the negative control, they were replaced by 200 µL PBS. The analysis was performed in triplicate.

Evaluation of genotoxicity

Comet assay

The Comet assay was used to measure the single and double strand breaks in DNA (Singh *et al*., 1995). An Eppendorf tube containing 200 µL extract and 200 µL leukocyte suspension was prepared. All steps after blood collection were performed in the dark or under dimmed red light to prevent additional DNA damage.

The Comet assay was performed in triplicate, analyzing 100 nuclei per sample of each concentration studied. These 100 cells were then examined microscopically at 40x magnification and classified according to tail damage: 0 being no damage to 4 being maximum damage. The sum of these values was used to ascertain a damage index (0–400) for each treatment. The analysis was performed in triplicate.

Statistical analysis

Data were expressed as mean ± standard deviation. Concentrations of flavonoid and phenolic compounds obtained by HPLC were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey test. Other results were analyzed using one-way ANOVA followed by the Bonferroni test. Results were considered statistically significant when $p<0.05$. Statistical analysis was performed using the software GraphPadPrism 5.0.

RESULTS

Phytochemical analysis

Total flavonoid and total polyphenolic contents

Total flavonoid contents, expressed as microgram equivalents of quercetin per gram of extract sample, were 240 µg and 229 µg of quercetin/g for LAECs and Cs1, respectively. Total polyphenolic contents, expressed as microgram equivalents of gallic acid per gram of extract sample, were 425 µg and 470 µg of gallic acid/g for LAECs and Cs1, respectively.

HPLC / DAD

Analysis by *HPLC / DAD* showed that flavonoids (quercetin, rutin and kaempferol) and phenolic acids (gallic, chlorogenic, caffeic and ellagic) were present in both LAECSs and Cs1, and their amounts are shown

in Table III. The retention times (tRs) of identified compounds were: gallic acid (tR=9.93 min; peak 1), chlorogenic acid (tR = 19.36 min; peak 2), caffeic acid (tR=24.57 min; peak 3), ellagic acid (tR=30.19 min; peak 4), rutin (tR=37.41 min; peak 5), quercetin (tR=46.23 min; peak 6), and kaempferol (tR=54.13 min; peak 6) (Figure 1). Table IV shows the amounts of these compounds at each concentration used in *in vitro* tests. LAECs contained higher concentrations of these compounds except for quercetin.

In vitro toxicological and antioxidant evaluation

Cell viability test

The aim of cell viability testing (Figure 2) was to choose concentrations for the *in vitro* studies. Concentrations that maintained the cell viability above 85% were 50 µg/mL (92%), 10 µg/mL (98%), 5 µg/mL (99%), and 2 µg/mL (100%) for LAECs and 50 µg/mL (87%), 10 µg/mL (96%), 5 µg/mL (98%), and 2 µg/mL (99%) for Cs1.

Radical-scavenging capacity – DPPH assay

LAECs and Cs1 demonstrated significant radical scavenging activity in the DPPH assay (Figure 3). They exhibited a scavenging effect of 85.13% and 88.95% respectively at a concentration of 50 µg/mL. The inhibition rate was concentration-dependent, being 49.12%, 27.52%, and 13.32% for LAECs and 73.12%, 45.35%, and 21.36% for Cs1, both at concentrations of 10, 5 and 2 µg/mL, respectively. Dimethyl sulfoxide (DMSO) was only used to help dissolve Cs1, and it was included in the analysis

TABLE III - Composition of lyophilized aqueous extract of *C. speciosa* (LAECs) and raw ethanol extract (Cs1)

Compounds	LAECs		Cs1		LOD	LOQ
	mg/g	%	mg/g	%	µg/mL	µg/mL
Gallic Acid	8.67 ± 0.03 [a]	0.86	9.37 ± 0.01 [a]	0.93	0.015	0.049
Chlorogenic Acid	43.19 ± 0.01 [b]	4.31	16.43 ± 0.02 [b]	1.54	0.009	0.029
Caffeic Acid	41.70 ± 0.02 [b]	4.17	30.28 ± 0.02 [c]	3.02	0.024	0.078
Ellagic Acid	6.53 ± 0.02 [c]	0.65	3.56 ± 0.03 [d]	0.35	0.013	0.042
Rutin	19.82 ± 0.01 [d]	1.98	3.91 ± 0.01 [d]	0.39	0.027	0.090
Quercetin	22.39 ± 0.03 [e]	2.23	41.80 ± 0.01 [e]	4.18	0.019	0.063
Kaempferol	65.41 ± 0.02 [f]	6.54	46.32 ± 0.03 [f]	4.63	0.026	0.085

Results are expressed as mean ± standard deviation of three determinations. Means followed by different letters differ by Tukey test ($p < 0.05$). LOD - Limit of Detection; LOQ - Limit of Quantification.

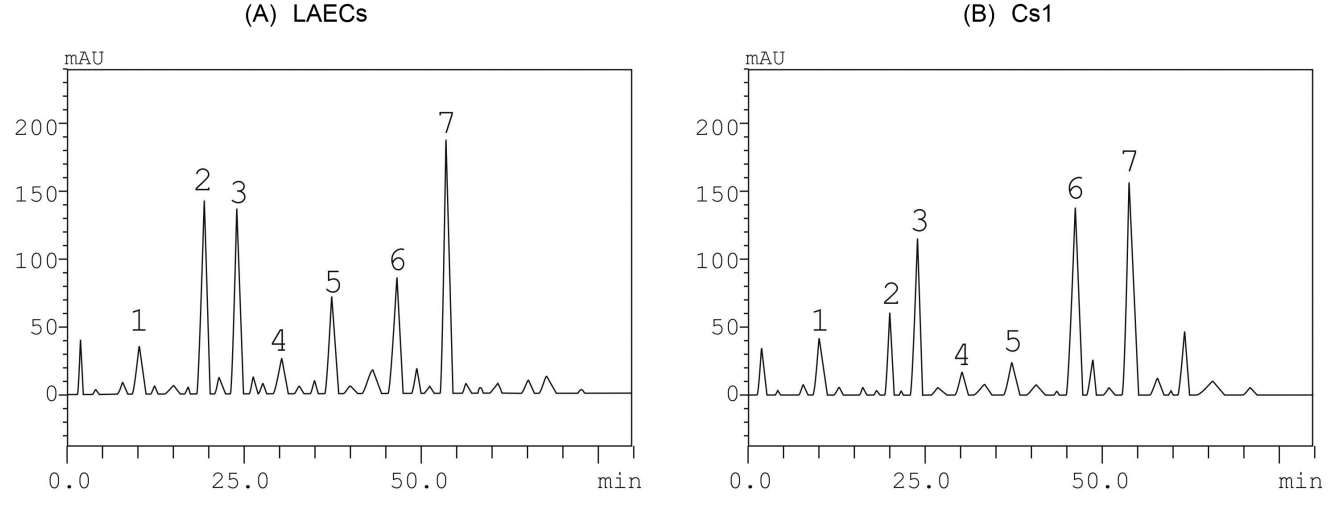

FIGURE 1 - Representative high performance liquid chromatography profiles of lyophilized aqueous extract of *C. speciosa* (LAECs) (A) and raw ethanol extract (Cs1) (B). UV detection at 325 nm. Gallic acid (1), chlorogenic acid (2), caffeic acid (3), ellagic acid (4), rutin (5), quercetin (6) and kaempferol (7).

TABLE IV - Composition of lyophilized aqueous extract of *C. speciosa* (LAECs) and raw ethanol extract (Cs1), at the concentrations used for the *in vitro* tests

Compound	LAECs				Cs1			
	µg/50 µg	µg/10 µg	µg/5 µg	µg/2 µg	µg/50 µg	µg/10 µg	µg/5 µg	µg/2 µg
Gallic Acid	0.43	0.09	0.043	0.02	0.47	0.09	0.05	0.02
Chlorogenic Acid	2.16	0.43	0.21	0.09	0.82	0.16	0.08	0.03
Caffeic Acid	2.08	0.42	0.21	0.08	1.51	0.30	0.15	0.06
Ellagic Acid	0.33	0.06	0.03	0.01	0.18	0.03	0.02	0.01
Rutin	0.99	0.20	0.09	0.04	0.19	0.04	0.02	0.01
Quercetin	1.12	0.22	0.11	0.04	2.09	0.42	0.20	0.08
Kaempferol	3.27	0.65	0.33	0.13	2.32	0.46	0.23	0.09

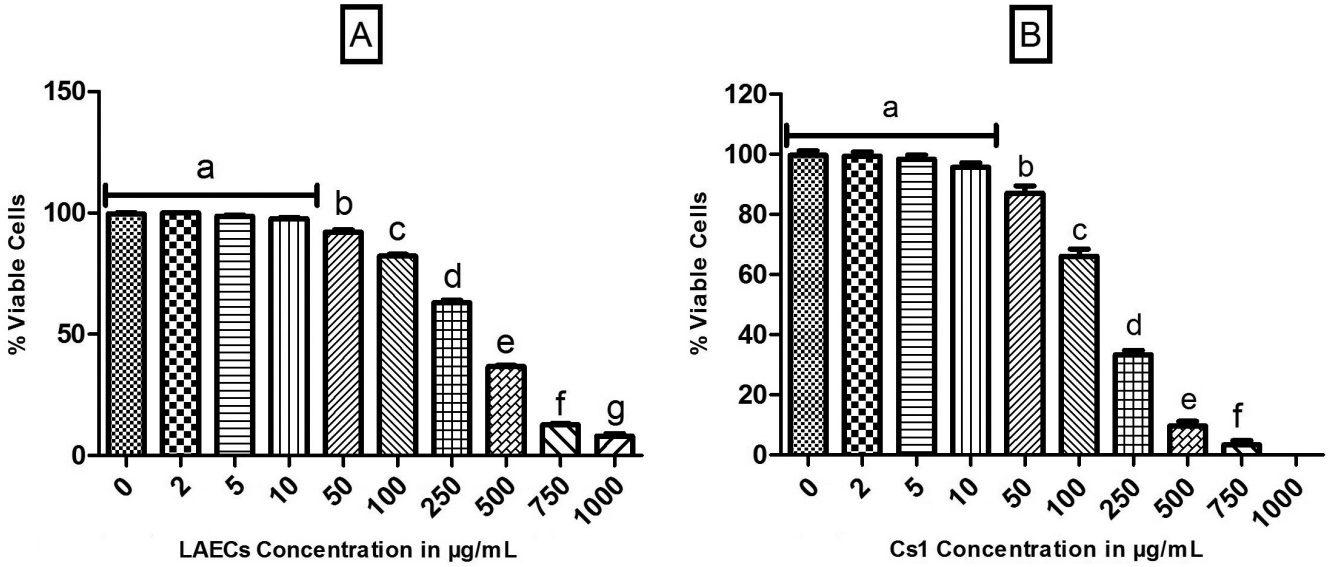

FIGURE 2 - Effects of lyophilized aqueous extract (LAECs) (A) and raw ethanol extract (Cs1) (B) on cell viability of human leukocytes. 0 µg/mL – Negative control (phosphate buffered saline). Data are expressed as mean ± SD. Columns with different letters are significantly different ($p<0.05$).

and figure to demonstrate that it did not have a statistically significant effect, and therefore no effect on the results.

Lipid peroxidation test

LAECs and Cs1 had an almost constant effect on lipid peroxidation at all concentrations tested, with similar effects to those of the negative control (Figure 4).

Determination of protein carbonyl groups

At concentrations of 50 and 10 µg/mL, LAECs demonstrated a carbonylation index statistically similar to that of the negative control (Figure 5); however, LAECs at 5 and 2 µg/mL and Cs1 at all concentrations showed results statistically similar to that of the positive control.

Evaluation of genotoxicity

Comet assay

Lyophilized aqueous extract of *C. speciosa* at concentrations of 10, 5, and 2 µg/mL and Cs1 at concentrations of 5 and 2 µg/mL produced statistically similar results to the negative control, showing no significant DNA damage; however, LAECs at 50 µg/mL and Cs1 at 50 and 10 µg/mL presented a score of 1 for DNA damage (Figure 6).

DISCUSSION

Studies of the toxicity and the protective effects of species used in traditional medicine are essential. In this

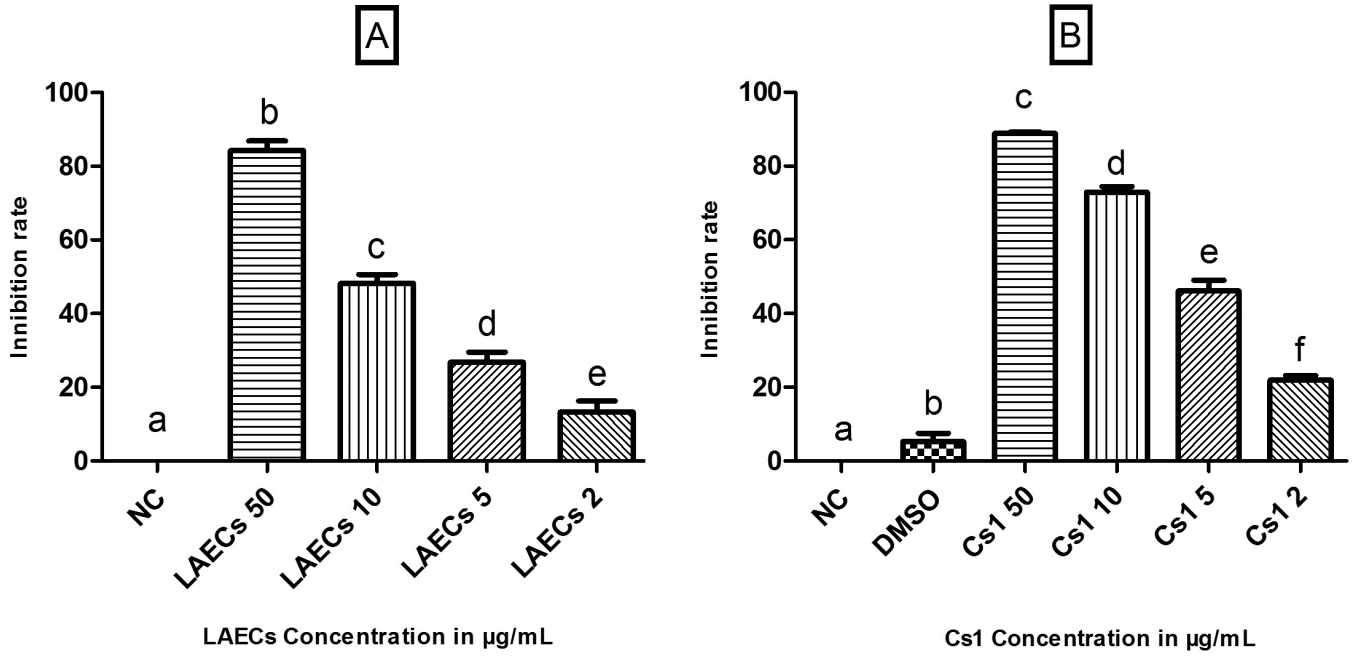

FIGURE 3 - 2,2-diphenyl-1-pricril-hydrazyl (DPPH) radical scavenging activity. (A) lyophilized aqueous extract (LAECs) at 50, 10, 5 and 2 µg/mL; (B) raw ethanol extract (Cs1) at 50, 10, 5 and 2 µg/mL; NC – Negative Control (distilled water); Data are expressed as mean ± SD. Columns with different letters are significantly different ($p<0.05$).

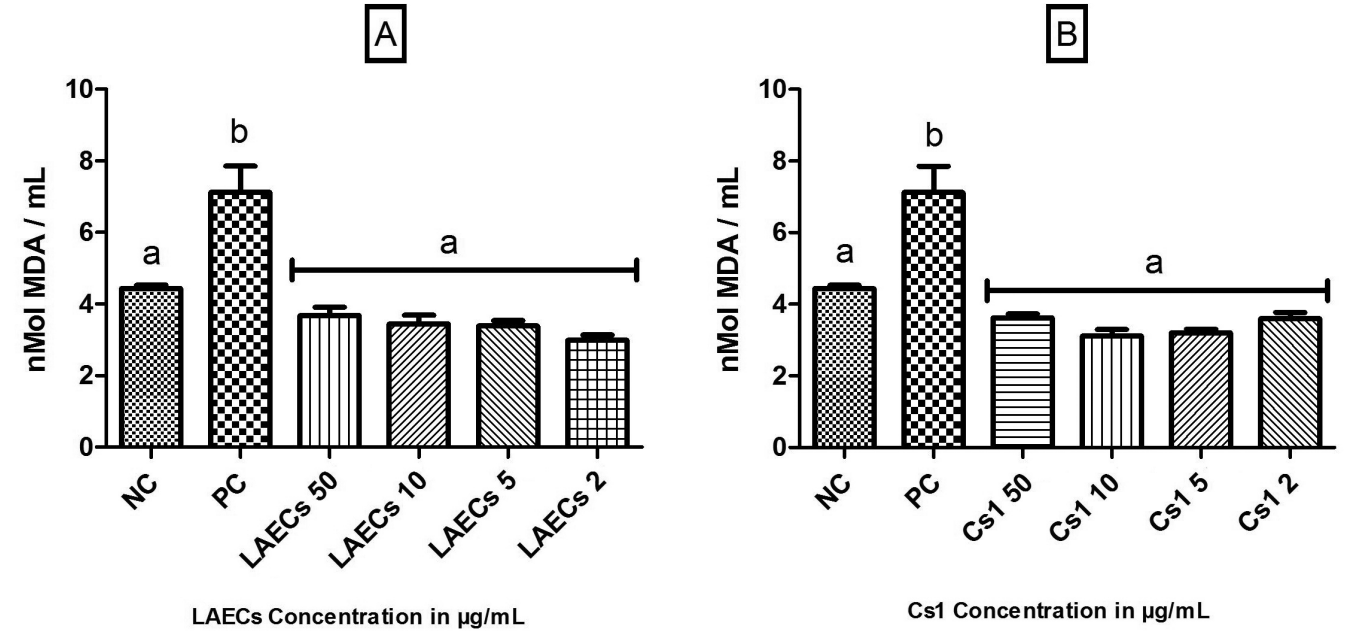

FIGURE 4 - Effects of lyophilized aqueous extract (LAECs) and raw ethanol extract (Cs1) on lipid peroxidation test induced by hydrogen peroxide (H_2O_2 100 µmol/L) in plasma. NC – Negative Control (phosphate buffered saline); PC – Positive Control; (H_2O_2 100 µmol/L); (A) LAECs (50, 10, 5 and 2 µg/mL); (B) Cs1 (50, 10, 5 and 2 µg/mL). Data are expressed as mean ± SD. Columns with different letters are significantly different ($p<0.05$).

sense, this work reports the phenolic composition of bark extracts of *C. speciosa* and their *in vitro* toxicity and antioxidant activity. The phytochemical analysis of Cs1 and LAECs showed a high concentration of polyphenolic compounds, comprising mainly flavonoids and phenolic acids. These compounds, which most commonly occur in

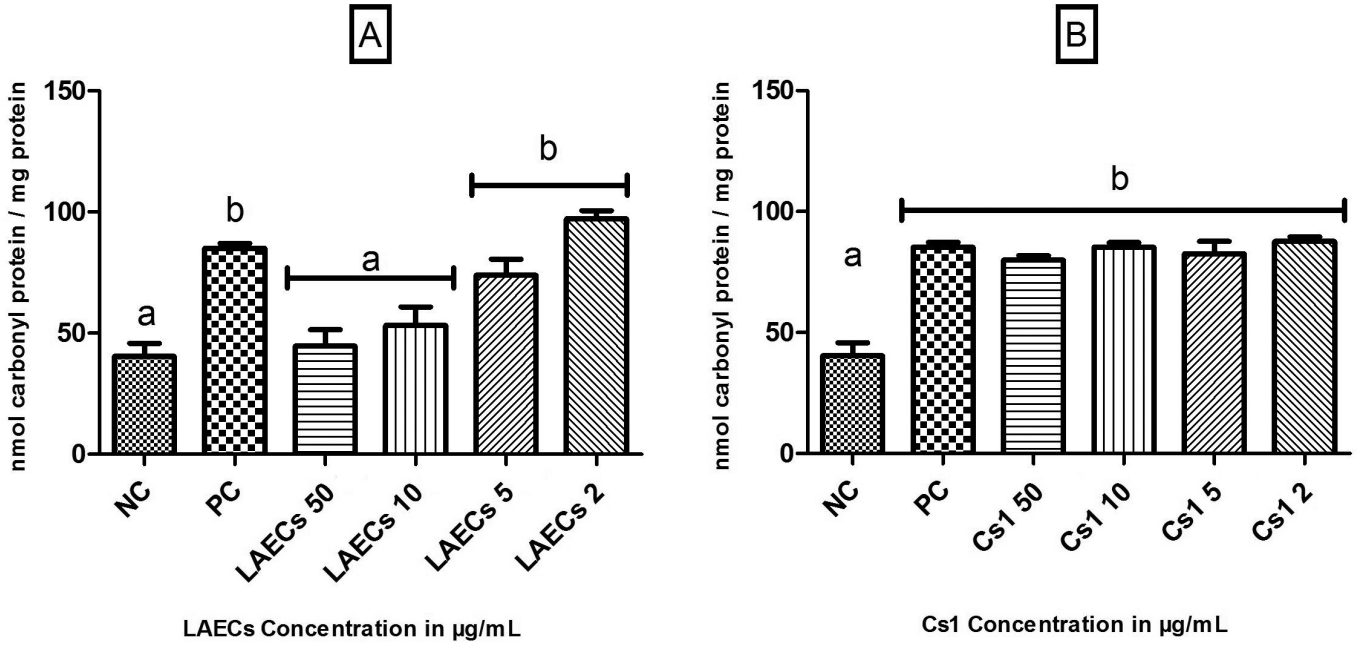

FIGURE 5 - Effects of lyophilized aqueous extract (LAECs) and raw ethanol extract (Cs1) on protein carbonylation induced by hydrogen peroxide (H_2O_2 100 µmol/L) in plasma. NC – Negative Control (phosphate buffered saline); PC – Positive Control; (H_2O_2 100 µmol/L[1]); (A) LAECs (50, 10, 5 and 2 µg/mL); (B) Cs1 (50, 10, 5 and 2 µg/mL). Data are expressed as mean ± SD. Columns with different letters are significantly different ($p<0.05$).

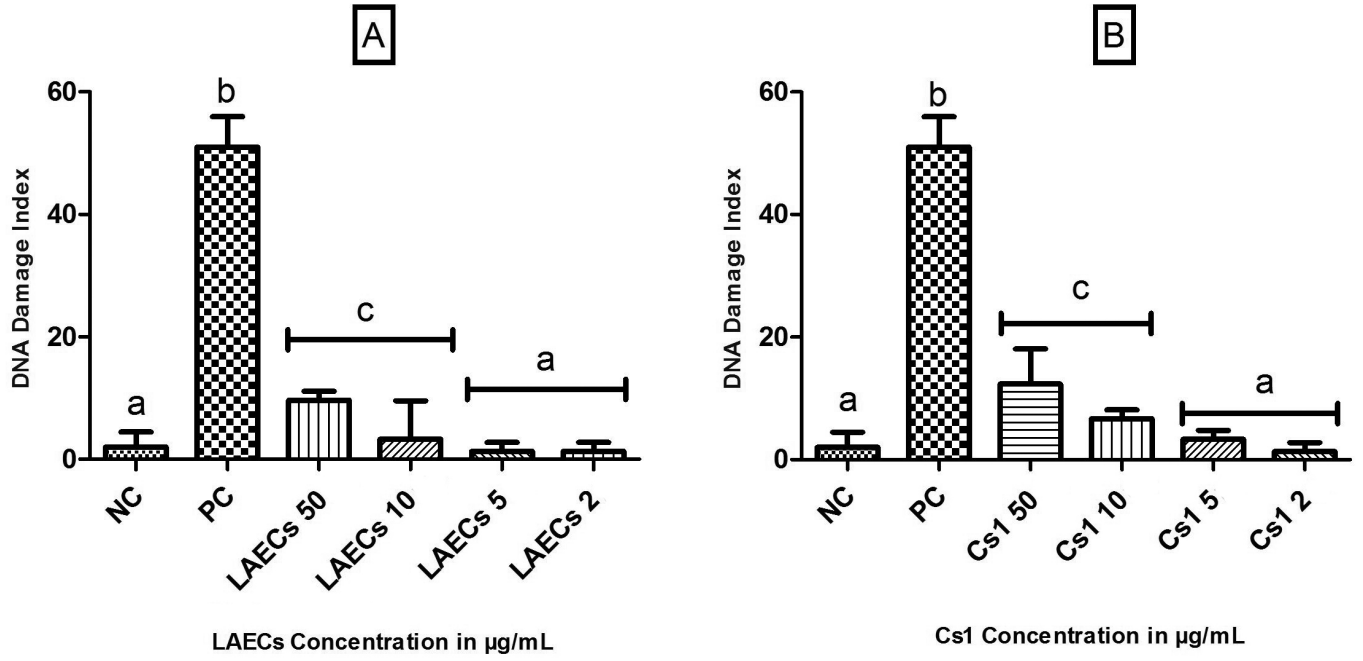

FIGURE 6 - Effects of lyophilized aqueous extract (LAECs) and raw ethanol extract (Cs1) in Comet assay of human leukocytes. NC – Negative Control (phosphate buffered saline, PBS); PC – Positive Control; (H_2O_2 100 µmol/L); (A) LAECs (50, 10, 5 and 2 µg/mL); (B) Cs1 (50, 10, 5 and 2 µg/mL). Data are expressed as mean ± SD. Columns with different letters are significantly different ($p<0.05$).

plants, represent an important class of phytochemical as they play a protective role against pathogens and predators. In humans they are known to have diverse physiological roles since they have antifungal, anti-inflammatory and antioxidant properties (Ayala-Zavala *et al*., 2012). Our results are consistent with those reported by Krishnaveni

et al. (2013), who found a high concentration of flavonoids (7.7 mg quercetin/g of extract) and phenolic compounds (3.3 mg gallic acid/g of extract) in *C. speciosa* leaves. LAECs showed higher concentrations of phenolic compounds than Cs1, with the exception of quercetin.

In the present study, a cell viability assay was performed to determine the concentrations to be used in the analysis (Koko *et al.*, 2008). The cell viability remained above 85% at relatively high concentrations, indicating that LAECs and Cs1 were non-toxic to human leukocytes. Antioxidant tests were performed using a DPPH assay; this is a widely accepted method for evaluation of *in vitro* antioxidant activity of extracts and is based on the capacity of antioxidant compounds present in the sample to capture DPPH radicals (Benhammou, Bekkara, Panovska, 2009; Ayala-Zavala *et al.*, 2012; Mohsin, Mahadevan, Kurup, 2014; Wan-Ibrahim, Sidik, Kuppusamya, 2010). The results showed that LAECs and Cs1 inhibited ≥ 50% of radicals at concentrations of 10 µg/mL and 50 µg/mL, showing that both samples appear to protect the studied biological matrix, since the concentrations tested were able to neutralize the radical DPPH. In a study by Ayala-Zavala *et al.* (2012), the authors examined several parameters to assess the antioxidant activity of *Phellinus gilvus*, *Phellinus rimosus*, and *Phellinus badius*, and found a correlation between the total polyphenolic content and DPPH radical scavenging activity; therefore, the inhibitory potential found in this work indicates an elevated concentration of these phytochemicals and their high antioxidant power.

Following determination of the antioxidant potential of *C. speciosa,* the *in vitro* effects of different concentrations of LAECs and Cs1 on some important parameters of oxidative stress were evaluated. An imbalance between production and inactivation of ROS can lead to oxidative stress and damage to various biological macromolecules, and proteins exposed to ROS can suffer oxidative modifications that can lead to changes in their functions, and, consequently, affect cellular metabolism (Gupta, Ballal, 2015). In this context, measurement of protein carbonyl groups in biological samples is a reliable parameter with which to assess ROS-mediated protein oxidation (Levine *et al*., 1990). In this study, LAECs at concentrations of 10 and 50 µg/mL showed a protein carbonyl content similar to the negative control, demonstrating no evidence of oxidative damage, and suggesting that these samples prevented H_2O_2-induced protein carbonylation. However, the results for LAECs at concentrations of 2 and 5 µg/mL were similar to those observed for the positive control, suggesting the occurrence of oxidative damage. At all concentrations tested, Cs1 demonstrated a carbonyl group content similar to the positive control, which indicates the occurrence of protein damage. These results may reflect the high concentrations of quercetin in Cs1 because, despite the beneficial actions of flavonoids, studies have demonstrated that quercetin has an *in vitro* pro-oxidant action (Choi, Chee, Lee, 2003; Heim, Tagliaferro, Bobilya, 2002; Yang *et al.*, 2012). Studies have reported that, in general, phenolic compounds exhibit pro-oxidant activity *in vitro* but not *in vivo*, and the conditions for this arise mainly in the presence of transition metals and acidic pH (Eghbaliferiz, Iranshahi, 2016). This suggests that further studies are necessary to determine whether these factors are present in our samples. In other species, ROS can target lipids and initiate the lipid peroxidation process, which may lead to molecular cell damage (Gupta & Ballal, 2015). In the present study, LAECs and Cs1 showed no differences compared to the negative control at all concentrations tested in the H_2O_2-induced lipid peroxidation assay, which indicates the absence of lipid oxidative damage and hence the potential antioxidant action of *C. speciosa*, consistent with the results of the DPPH test. These results are an important indication of the safety of this species, because the occurrence of lipid peroxidation is directly related to various disease processes such as carcinogenesis and atherosclerosis.

Besides protein and lipids, DNA is also a significant target for oxidative damage mediated by ROS. Samples were subjected to the Comet assay, which is considered a sensitive method for the detection of single and double DNA strand breaks (Collins *et al.,* 2008). LAECs at concentrations of 2, 5 and 10 µg/mL, and Cs1 at concentrations of 2 and 5 µg/mL, showed no significant DNA damage. Both samples at higher concentrations (50 µg/mL of LAECs and 10 and 50 µg/mL of Cs1) showed low levels of DNA damage (score 1). These results indicate that further genotoxic studies are required in order to deepen knowledge about the safety of *C. speciosa*.

CONCLUSION

This study revealed that *C. speciosa* extracts have promising antioxidant potential, which may be related to their high polyphenolic content. Phenolic compounds are the constituents responsible for the antioxidant potential of natural products (Krishnaveni *et al*., 2013; Loganayaki, Siddhuraju, Manian, 2013) from species of the genus *Passiflora* and *Citrus*, both known for being rich in flavonoids and with well-established antioxidant activity. Furthermore, LAECs and Cs1 showed low toxicity *in vitro*, especially at a concentration of 10 µg/mL. *C. speciosa*

extracts showed either no damage or at the most a low degree of DNA damage (grade 1) in the Comet assay and they also prevented lipid peroxidation at all concentrations tested. Aqueous extract (LAECs), which is used in folk medicine, showed no damage to proteins at concentrations of 10 and 50 μg/mL.

The use of plants is widely accepted by the public, and this fact is closely related to the popular belief that natural products are free of adverse effects. However, the popularization of the use of medicinal plants, their ease of access, and lack of professional guidance on their correct use, represent a health risk (Al-Arifi, 2013; Jeong *et al*., 2012). This study describes a preliminary evaluation of the biological activity and safety of *C. speciosa* and shows that this species exhibits *in vitro* antioxidant potential and low toxicity, which may be related at least in part to its high concentration of polyphenols. The results provide an important foundation for knowledge about this species, which is popularly used for the reduction of serum cholesterol, triglyceride and glucose levels. Further phytochemical analyses are necessary to determine the chemical compounds responsible for the antioxidant activity *of C. speciosa* and to correlate them with other possible pharmacological or even toxicological effects of this very promising plant.

ACKNOWLEDGEMENTS

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS). The authors thank the researchers of Laboratório de Biotecnologia da Reprodução (Biotech – UNIPAMPA) for their collaboration in the experiments.

REFERENCES

AL-ARIFI, M.N. Availability and needs of herbal medicinal information resources at community pharmacy, Riyadh region, Saudi Arabia. *Saudi Pharm J*. v.21, n.4, p.351-360, 2013.

AYALA-ZAVALA, J.F.; SILVA-ESPINOZA, B.A.; CRUZ-VALENZUELA, M.R.; VILLEGAS-OCHOA, M.A.; ESQUEDA, M.; GONZÁLEZ-AGUILAR, G.A.; CALDERÓN-LÓPEZ, Y. Antioxidant and antifungal potential of methanol extracts of *Phellinus spp*. from Sonora, Mexico. *Rev. Iberoam. Micol*., v.29, n.3, p.132-138, 2012.

BALICK, M.J.; COX, P.A. *Plants, people and culture*: the science of ethnobotany. New York: HPHLP, 1996. 228p.

BELESKI-CARNEIRO, E.; SUQUI, J.; REICHER, F. Structural and biological features of a hydrogel from seed coats of *Chorisia speciosa. Phytochemistry*., v.61, n.2, p.157-163, 2002.

BENHAMMOU, N.; BEKKARA, F.A.; PANOVSKA, T.K. Antioxidant activity of methanolic extracts and some bioactive compounds of *Atriplex halimus*. *Comptes Rendus Chimie*, v.12, n.12, p.1259-1266, 2009.

BOLIGON, A.A.; FELTRIN, A.C.; MACHADO, M.M.; JANONIK, V.; ATHAYDE, M.L. HPLC analysis and phytoconstituents isolated from ethyl acetate fraction of *Scutia buxifolia* Reiss. leaves. *Lat. Am. J. Pharm*., v.28, n.1, p.121-124, 2009.

BRASIL. Ministério da Saúde. *Política nacional de plantas medicinais e fitoterápicos*. Brasília: Ministério da Saúde, 2007. 60p. Disponível em: <http://bvsms.saude.gov.br/bvs/publicacoes/politica_nacional_fitoterapicos.pdf>. Access: 19 Set. 2016.

BRASIL. Ministério da Saúde. *RENISUS - Relação Nacional de Plantas Medicinais de Interesse ao SUS*. Brasília: Secretaria de Ciência, Tecnologia e Insumos Estratégicos, 2009. 3p. Disponível em: <http://bvsms.saude.gov.br/bvs/sus/pdf/marco/ms_relacao_plantas_medicinais_sus_0603.pdf>. Access: 19 Set. 2016.

BUROW, M.E.; WELDON, C.B.; TANG, Y.; NAVAR, G.L.; KRAJEWSKI, S.; REED, J.C.; HAMMOND, T.G.; CLEJAN, S.; BACKMAN, B.S. Differences in susceptibility to tumor necrosis factor α-induced apoptosis among MCF-7 breast cancer cell variants. *Cancer Res*., v.58, n.21, p.4940-4946, 1998.

CALIXTO, J.B. Twenty-five years of research on medicinal plants in Latin America: a personal review. *J. Ethnopharmacol*., v.100, n.1-2, p.131-134, 2005.

CAPPELATTI, L.; SCHMITT, L.J. Caracterização da flora arbórea de um fragmento urbano de floresta estacional semidecidual no Rio Grande do Sul, Brasil. *Pesq. Botânica*, v.60, p.341-354, 2009.

CARLINI, E.A.; RODRIGUES, E.; MENDES, F.R.; TABACH, R.; GIANFRATTI, B. Treatment of drug dependence with Brazilian herbal medicines. *Rev. Bras. Farmacogn*., v.16, p.690-695, 2006.

CARTAXO, S.L.; SOUZA, M.M.A.; ALBUQUERQUE, U.P. Medicinal plants with bioprospecting potential used in semi-arid northeastern Brazil. *J. Ethnopharmacol.*, v.131, n.2, p.326-342, 2010.

CHANDRA, S.; MEJIA, E.G. Polyphenolic compounds, antioxidant capacity, and quinone reductase activity of an aqueous extract of *Ardisia compressa* in comparison to mate (*Ilex paraguariensis*) and green (*Camellia sinensis*) teas. *J. Agr. Food Chem*., v.52, n.11, p.3583-3589, 2004.

CHOI, E.J.; CHEE, K.M.; LEE, B.H. Anti- and prooxidant effects of chronic quercetin administration in rats. *Eur. J. Pharmacol*., v.482, n.1-3, p.281-285, 2003.

COLLINS, A.R.; OSCOZ, A.A.; BRUNBORG, G.; GAIVÃO, I.; GIOVANNELLI, L.; KRUSZEWSKI, M.; SMITH, C.C.; STETINA, R. The comet assay: topical issues. *Mutagenesis*, v.23, n.3, p.143-151, 2008.

EGHBALIFERIZ, S.; IRANSHAHI, M. Prooxidant activity of polyphenols, flavonoids, anthocyanins and carotenoids: Updated review of mechanisms and catalyzing metals: prooxidant activity of polyphenols and carotenoids. *Phytother Res*., v.30, n.9, p.1379-91, 2016.

EL-SHENAWY, N.S.; MOHAMMADDEN, A.; AL-FAHMIE, Z.H. Using the enzymatic and non-enzymatic antioxidant defense system of the land snail *Eobania vermiculata* as biomarkers of terrestrial heavy metal pollution. *Ecotox. Environ. Safe*., v.84, p.347-354, 2012.

GAUR, K.; KORI, M.L.; TYAGI, L.K.; NEMA, R.K.; SHARMA, C.S.; TRIPATHI, P. In-vitro antioxidant activity of leaves of *Ipomoea fistulosa* Linn. *Acad. J. Plant. Sci*., v.2, n.2, p.60-64, 2009.

GUPTA, A.; BALLAL, A. Unraveling the mechanism responsible for the contrasting tolerance of *Synechocystis* and *Synechococcus* to Cr(VI): Enzymatic and non-enzymatic antioxidants. *Aquat. Toxicol*., v.164, p.118-125, 2015.

HEIM, K.E.; TAGLIAFERRO, A.R.; BOBILYA, D.J. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem*., v.13, n.10, p.572-584, 2002.

JEONG, T.Y.; PARK, B.K.; CHO, J.H.; KIM, Y.I.; AHN, Y.C.; SON, C.G. A prospective study on the safety of herbal medicines, used alone or with conventional medicines. *J. Ethnopharmacol*., v.143, n.3, p.884-888, 2012.

KHARRAZI, H.; VAISI-RAYGANI, A.; RAHIMI, Z.; TAVILANI, H.; AMINIAN, M.; POURMOTABBED, T. Association between enzymatic and non-enzymatic antioxidant defense mechanism with apolipoprotein E genotypes in Alzheimer disease. *Clin. Biochem*., v.41, n.12, p.932-936, 2008.

KOKO, W.S.; MESAIK, M.A.; YOUSAF, S.; GALAL, M.; CHOUDHARY, M.I. In vitro immunomodulating properties of selected Sudanese medicinal plants. *J. Ethnopharmacol*., v.118, n.1, p.26-34, 2008.

KRISHNAVENI, M.; AMSAVALLI, L.; CHANDRASEKAR, R.; MADHAIYAN, P.; DURAIRAJ, S. Antioxidant activity of plants at Govt. College of Engineering Campus, Salem, Tamil nadu, India. *Int. J. Pharm. Sci. Rev. Res*., v.21, n.1, p.160-163, 2013.

LADEJI, O.; OMEKARAH, I.; SOLOMON, M. Hypoglycemic properties of aqueous bark extract of *Ceiba pentandra* in streptozotocin-induced diabetic rats. *J. Ethnopharmacol*., v.84, n.2-3, p.139-142, 2003.

LEVINE, R.L.; AMSAVALLI, L.; CHANDRASEKAR, R.; MADHAIYAN, P.; DURAIRAJ, S. Damage to proteins and lipids tissues under oxidative stress. *Method Enzymol.*, v.186, p.464-478, 1990.

LOGANAYAKI, N.; SIDDHURAJU, P.; MANIAN, S. Antioxidant activity and free radical scavenging capacity of phenolic extracts from *Helicteres isora* L. and *Ceiba pentandra* L. *J. Food Sci. Tech.*, v.50, n.4, p.687-695, 2013.

MASHWANI, Z.U.; KHAN, M.A.; ULLAH, Z.; CHAUDHARY, H.J. An ethno botanical perspective of traditional medicinal plants from the Khattak tribe of Chonthra Karak, Pakistan. *J. Ethnopharmacol.*, v.165, p.251-259, 2015.

MEDEIROS, P.M.; LADIO, A.H.; ALBUQUERQUE, U.P. Sampling problems in Brazilian research: a critical evaluation of studies on medicinal plants. *Rev. Bras. Farmacogn*., v.24, n.2, p.103-109, 2015.

MOHSIN, S.; MAHADEVAN, R.; KURUP, G.M. Free-radical-scavenging activity and antioxidant effect of ascophyllan from marine brown algae *Padina tetrastromatica*. *Biom. Prev. Nutr*., v.4, n.1, p.75-79, 2014.

MORABITO, F.; CRISTIANI, M.; SAIJA, A.; STELITANO, C.; CALLEA, V.; TOMAINO, A.; MINCIULLO, P.L.; GANGEMI, S. Lipid peroxidation and protein oxidation in patients affected by Hodgkin's Lymphoma. *Mediat. Inflamm*., v.13, n.5/6, p.381-383, 2004.

OHKAWA, H.; OHISHI, H.; YAGI, K. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. *Ann. Clin. Biochem*., v.95, n.2, p.351-358, 1979.

PEREIRA, L.P.; SILVA, F.E.B.; FLORES, E.M.M.; SCHREKKER, H.S.; MACHADO, M.M.; OLIVEIRA, L.F.S. *In vitro* ZnCl2 cytotoxicity and genotoxicity in human leukocytes: Zero-order kinetic cellular zinc influx. *Acta Scient. Health Sci.*, v.37, n.1, p.63-68, 2015.

SAID, W.M.; NAHLA, O.M.; EHSAN, N.S.K. Comparative study of three species of Malvatheca (Bombacoideae) and Malvoideae (Malvaceae *sensu lato*) using morphological, anatomical and RAPD-PCR analyses. *Adv. Environ. Biol*., v.7, n.2, p.415-426, 2013.

SCARPA, G.F. Medicinal plants used by the Criollos of Northwestern Argentine Chaco. *J. Ethnopharmacol*., v.91, n.1, p.115-135, 2004.

SHARMA, O.P.; BHAT, T.K. DPPH antioxidant assay revisited. *Food Chem*., v.113, n.4, p.1202-1205, 2009.

SINGH, N.; MCCOY, M.; TICE, R.; SCHNEIDER, E. A simple technique for quantification of low levels of DNA damage in individual cells. *Exp. Cell Res*., v.175, n.1, p.184-191, 1995.

VELOSO, D.P.; PAULA, V.F.; BARBOSA, L.C.A.; DEMUNER, A.J. A química da família Bombacaceae. *Quím Nova*., v.20, n.6, p.627-630, 1997.

WAN-IBRAHIM, W.I.; SIDIK, K.; KUPPUSAMYA, U.R. A high antioxidant level in edible plants is associated with genotoxic properties. *Food Chem*., v.122, n.4, p.1139-1144, 2010.

YANG, B.; CHEN, F.; HUA, Y.; HUANG, S.S.; LIN, S.; WEN, L.; JIANG, Y. Prooxidant activities of quercetin, p-courmaric acid and their derivatives analysed by quantitative structure-activity relationship. *Food Chem*., v.131, p.508-512, 2012.

ZHISHEN, J.; MENGCHENG, T.; JIANMING, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*., v.64, n.4, p.555-559, 1999.

Received for publication on 24th May 2016
Accepted for publication on 25th November 2016