

Ripe fruits of *Bromelia antiacantha*: investigations on the chemical and bioactivity profile

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RESUMO: “Química e atividade *in vitro* da banana-do-mato (*Bromelia antiacantha* Bertol., Bromeliaceae)”. O chá dos frutos maduros de *Bromelia antiacantha* (banana-de-bugre; banana-do-mato) é usado tradicionalmente para tratar distúrbios respiratórios, como antitussígeno e também para tratar úlceras de pele e boca. Neste trabalho pesquisou-se o potencial de captação de radicais livres do extrato aquoso e extrato metanólico através da reação de redução do DPPH e do fosfomolibdenio, sendo encontrada baixa atividade nos dois modelos (abaixo de 5 mg/ml nenhuma atividade foi detectada em ambos extratos). Os compostos fenólicos totais foram determinados através de Folin-Ciocalteu, sendo encontrados 500 µg/g de fruto maduro fresco, e foi desenvolvido *fingerprint* dos compostos fenólicos através de CLAE-DAD. Nenhuma citotoxicidade foi observada em fibroblastos L929 para extratos aquoso, metanólico e lipídico entre 500 e 0,01 µg/ml, assim como baixa genotoxicidade (1000 µg/mL) quando comparados aos controles negativo e positivo (MMS). Abundantes cristais de oxalato de cálcio e de sacarose foram observados e caracterizados por RMN e difração de Raios-X respectivamente, e a composição centesimal do fruto indicou abundantes carboidratos (45%) e lipídeos (18%). A análise de carboidratos encontrou componentes monossacarídicos ácidos e foram encontrados ácido palmítico e linoléico em quantidades similares (30% cada) e 20% de ácido oléico, entre outros ácidos graxos através de CG-DIC. A viscosidade da sub-fração polissacarídica solúvel em água (5 g/L) foi semelhante a da água.

Unitermos: *Bromelia antiacantha*, antioxidante, citotoxicidade, genotoxicidade, carboidratos, ácidos graxos.

ABSTRACT: *Bromelia antiacantha* (known as wild banana) is traditionally used to treat coughs, as an emollient and antitussive, and to treat mouth and skin ulcers. To investigate the radical scavenger properties, DPPH and phosphomolybdenum were used, with low antioxidant activity being observed for both (below 5 mg/ml of extract presented no activity for both techniques). An HPLC fingerprint of phenolic compounds was developed and total phenolics through Folin-Ciocalteu method was an average of 500 µg/g of fresh fruits. Any cytotoxicity was observed on L929 fibroblasts cells for aqueous, methanol and lipid extracts between 500 and 0.01 µg/ml. The wild banana extract (1000 µg/mL) shown a lower genotoxicity when compared to the negative and positive (MMS) controls. Abundant calcium oxalate crystals and sucrose were found and characterized through NMR and X-ray diffraction respectively. Its centesimal composition indicated abundant carbohydrates (45%) and lipids (18%). The carbohydrate analysis showed acidic monosaccharides, and the lipid analysis shown palmitic and linoleic acids in similar quantities (30% each) and oleic acid 20%, among others, through GC-FID. The viscosity of the water-soluble polysaccharide sub-fraction (5 g/L) was similar to that of water.

Keywords: *Bromelia antiacantha*, antioxidant, cytotoxicity, carbohydrate, fatty acids, genotoxicity.

INTRODUCTION

Bromeliaceae is the largest family of plants in the Atlantic forest, with a high degree of endemism (Canela & Sazima, 2003). *Bromelia antiacantha* Bertol., is a synonym of *Agallostachys antiacantha* (Bertol.) Beer, and are also classified as *Bromelia fastuosa* Lindl. and *Agallostachys fastuosa* (Lindl.) Beer (<http://mobot.mobot.org/W3T/Search/vast.html>). This species produces abundant fruits with pungent, astringent taste,

known as Wild banana (Biavatti et al., 2007). The first inhabitants of the region (the Bororos Indians) boiled the fruits to treat coughs, and used as an emollient, a tradition which was continued by the European colonizers and exists until the present day (Reiz, 1983). The fruits can be eaten without ill effects, other than mild irritation around the mouth and lips. This is apparently the result of a combination of mechanical irritation caused by calcium oxalate raphides and subcutaneous injection of a proteolytic enzyme (Payrol et al., 2005).

There have been no scientific studies that characterize the properties or the composition of Wild banana. From ripe fruits of *B. antiacantha* a cysteine protease was recently characterized. By their action as anti-inflammatory agents and increasing the permeability of the blood-brain barrier to nutrients and therapeutic agents, plant cysteine proteases, have shown certain perspective application to Alzheimer's disease (Valles et al., 2007).

From the green fruits of *B. balansae*, an endopeptidase enzyme was isolated (Pardo et al., 2000). The Bromeliaceae family produces large quantities of proteinases, and the isolation of new proteases from plants is of use to the food and cosmetics industries (Pardo et al., 2001).

From the extracts of the fruits of *B. plumieri*, glucopyranosyl anthranilate was isolated, a flavoring precursor (Parada et al., 1996). The methanol extract of the pulp of the fruits of *B. pinguin* showed antifungal activity against some strains of *Trycophyton* (Camacho-Hernandez et al., 2002). A non-proteic extract of the fruits from this same species inhibited uterine motility in rats (Matadial et al., 1999). From the same species, some preliminary results, such as anti-helminthic and antimicrobial activities have been published (Abreu Payrol et al., 2001a). Some simple organic acids have also been detected in its fruits (glycolic, malic, and lactic acid) (Abreu Payrol et al., 2001b), through HPLC, and for CG-MS some fatty acids have been described (Abreu Payrol et al., 2001c).

This work involves an investigation of the medicinal extracts of ripe fruits of *B. antiacantha* in relation to its antioxidant, cytotoxic and genotoxic potentials *in vitro*; determination of the total phenolics and its chromatographic profile, its centesimal composition, fatty acids and carbohydrate composition and rheological properties.

MATERIAL AND METHODS

Plant material

After fruitification of *B. antiacantha*, 4 kg of its ripe fruits were collected in the Vale do Itajaí region by Epagri (Santa Catarina Agricultural Research Company), and its botanical identity confirmed by Agronomical Engineer Antonio Amaury da Silva.

Preparation of extracts

The fruits (1 kg) were opened and immediately boiled in potable water. Approximately 2 liters of aqueous extract was subsequently lyophilized, yielding 20 g of extracted residue. In addition, a methanol extract was prepared by exhaustive maceration of the ground fruits (763 g) which were then dried in a rotary evaporator, yielding 250 g of extractable solids. After

a determined time, there was spontaneous formation of abundant sucrose crystals which were recrystallized and analyzed by ^1H and ^{13}C NMR and compared with published data.

Chromatographic analysis

A Waters Liquid chromatography system (HPLC) was used, consisting of a 600 pump, Photodiode Array Detector (PDA), Millennium software and manual Rheodyne injector. All the solvents used were HPLC grade, filtered (0.45 μm) and sonicated under vacuum. The samples were properly diluted and filtered through a regenerated cellulose membrane (0.45 μm).

All the analyses were carried out in triplicate in a Novapack C18 column (5 μm , 3.5 x 100 mm) thermostated (30 $^\circ\text{C}$), using a gradient system comprised of water acidified with 0.05% acetic acid (A) and methanol (B) 40 minutes - 100% of A up to 20%, flow 0.8 mL/min.

Determination of the total phenolics

The total phenolics content was determined using the Folin-Ciocalteu method (Singleton et al., 1999), with the following modifications: 0.1 ml solvent or sample, 7.9 ml water, 0.5 ml FC reagent, and 1.5 ml Na_2CO_3 (7.5%) were mixed and allowed to stand for 30 min at room temperature. Absorption was measured at 765 nm in a UV-Vis double-array spectrophotometer SHIMADZU UV - 1601. The results were expressed as one microgram of gallic acid equivalent (GAE) per milliliter of solution.

Antioxidant activity

The radical scavenger potential of the ripe fruits of *B. antiacantha* was verified through two spectrophotometric models *in vitro*: 1. a reduction in DPPH (2,2-difenyl-1-picrylhydrazyl) (Brand-Williams et al., 1995) and 2. the formation of the phosphomolybdenum complex (Prieto et al., 1999), also based on the change in absorption after reduction of the molybdenum (VI) to molybdenum (V).

In both methods, the extract samples were prepared in a series of dilutions, in appropriate solvent, mixed with the reagent in question, and after a period of incubation, the absorbance was measured removing using a Shimadzu UV-Vis 1601 spectrophotometer. The antioxidant capacity is based on a comparison of the molar absorption coefficient of the blank compared with the samples, expressed as a percentage, using the standard antioxidant substances: rutin and tocopherol, respectively. All the tests were carried out in triplicate.

Cytotoxic activity - Fibroblast L929

Cell culture experiments were carried out using L929 mouse fibroblasts (BCRJ CR020/ATCC CCL1). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) and 2mM glutamine at 37°C, 5,0% CO₂, and 95% relative humidity.

Cell viability was evaluated using the MTT assay. Briefly, L929 cells were inoculated into 96-well microtiter plates at a density of 20.000 cells/well. After 24 h the culture medium was replaced with serial dilutions from the extract stock solutions in FCS-free DMEM. After an incubation period of 1, 24, 48 and 72 h, 20 µL MTT (5 mg/ml in PBS) were added. After an incubation time of 4 h unreacted dye was removed by aspiration and the purple formazan product was dissolved in 200 µL/well dimethylsulfoxide (Merck) and quantified by a plate reader (Tecan GENios Pro) at 570 nm. Relative cell viability [%] related to control wells containing cell culture medium without extract was calculated.

Genotoxicity

In the comet assay, cells were obtained from Swiss mouse medulla, and were embedded in low melting agarose (1% in PBS), in slide glasses previously recovered with normal point melting agarose (1% in PBS). The slides were placed in a chilled lysing solution (2.5 M NaCl, 100 mM Na₂EDTA, 10 mM Tris-HCl, 1% sarkosyl, 10% DMSO and 1% Triton X-100, pH 10) and kept at 0°C in the dark for 60 min. Electrophoresis was done at pH 13 to detect double strand breaks, single strand breaks of DNA and alkali-labile sites. Electrophoresis was done in 300 mM NaOH and 1 mM Na₂EDTA (pH 13) at 5°C in the dark for 30 min at 25V (0.96 V/cm) and approximately 300 mA after alkali treatment with electrophoresis buffer at 5°C in the dark for 30 min. All slides were neutralized by 400 mM Tris (pH 7.5), and stained with 50 µL of 20 µg/mL ethidium bromide. The migration of nuclear DNA from the cells was measured for 50 cells at concentration of 1000 µg/mL, and the mean migration of 50 nuclei was calculated in each trial (Itoh et al., 2006).

Analysis of centesimal composition (A) and carbohydrates (B) of the fruits

A. The centesimal composition was determined following AOAC (1995) for Moisture, Ashes, Lipids, Proteins and Carbohydrates.

B. The *B. antiacantha* fruits were ground, and submitted to an exhaustive lipid extraction with ether in Soxhlet apparatus. The extract obtained was used to fatty acid analysis, and the residue was exhaustively extracted

in blender with water at 25 °C. The water soluble fraction was denominated PS. The residue of non-water soluble polysaccharides were extracted using NaOH 0.1 N/Borate at 50 °C. The polysaccharides obtained were denominated total hemicellulose (HT). The non-water non-alkaline aqueous soluble polysaccharides are denominated lignocelluloses (LC).

The total content of carbohydrates, uronic acids and proteins

The total carbohydrate was assayed by the phenol-sulphuric acid method, as described by Dubois et al. (1956), with some modifications. The uronic acids were determined by the *m*-hydroxybiphenyl method (Blumenkrantz and Asboe Hansen, 1973). The proteins were determined by the Bradford method (1976).

Determination of the monosaccharide composition

Monosaccharide contents of the polysaccharide fractions were determined through: Hydrolysis with sulphuric acid (Selvendran et al., 1979) and reduction, acetylation and analysis by gas chromatography.

The monosaccharides resulting from the hydrolysis were reduced in the presence of sodium borohydride (NaBH₄), at a temperature of 50 °C, for 12 hours. The reaction was neutralized by the addition of diluted acetic acid and the excess sodium ions were removed by the addition of Lewatt cationic resin S-100 (H⁺), followed by filtration and drying in a rotary evaporator. The residue was then washed with methanol, to form trimethyl borate, which is distilled with the excess methanol. The dry alditols were then acetylated in the presence of acetic anhydride-pyridine (1:1, v/v), at room temperature for 15 hours. The reaction was interrupted by the addition of ice, the residual pyridine removed by the addition of copper sulphate, and the alditols acetate extracted with chloroform. The products were analyzed by CG according to Wolfrom and Thompson (1963), in gas chromatography HP model 5890 series 2, with detector and injector at 250 °C and capillary column DB-225 at 220 °C, using nitrogen as the carrier gas.

Analysis of the fatty acids

The lipid extract (50 µL) obtained with ether in a Soxhlet apparatus was treated with 500 µL of KOH 0.5 M in MeOH, for 90 min in a water-bath at 60 °C. After cooling, 1.5 ml of a methylation solution was added (6 mL of sulfuric acid in 120 mL of MeOH), and it was maintained at 60 °C for 90 min. The solution was extracted with 2 ml of hexane (Hartman and Lago, 1973). The hexane solution was filtered (0.45 µm) and

injected (1 μ l), for analysis through CG/FID (Shimadzu CG17A, automatic injector AOC20I) using SP2340 [100% poly(biscyanopropyl siloxane) - 60 m x 0.25 mm id x 0.2 μ m] column with an initial temperature program of 120 °C /5 min then 4 °C/min until 240 °C, maintained for 25 min. Helium was used as the carrier gas, at 1.7 ml/min. The injector and detector temperature were 250 and 260 °C, respectively. The peaks for the standards (kit FAME MIX, 37 components, Sigma-Aldrich) were identified by the retention times obtained through comparison of the standards with sample.

Rheological analysis

The aqueous extract of the subfraction of water-soluble polysaccharides was analysed for its rheological property. Non-oscillatory rheological analyses were performed with a Haake VT550, sensor PK1o. The temperature of the system was controlled at 25 °C with a Haake DC30 circulatory bath.

Crystallographic analysis

The fresh fruits (4 g) were soaked in distilled water and then filtered (0.04 μ m). The residue obtained was deposited over glass laminas. All these procedures were monitored using a binocular lens (Leica CME, 40x) in order to be sure that collection of crystals was properly done.

For X-ray characterization, was used a Rigaku-Miniflex X-ray powder diffraction apparatus, using the Cu K α (radiation) (Department of Physics, Universidade Federal de Santa Catarina - UFSC).

Statistical analysis

All data were obtained at least in triplicate and presented as means and standard deviation. One-way ANOVA at a significance level of $P \leq 0.05$. To compare results within one group, Tukey post-tests were applied.

RESULTS AND DISCUSSION

Rubbing of the fresh ripe fruit on a slide analyzed under a microscope, abundant raphides of calcium oxalate can be seen. Oxalate-producing plants, which include many crop plants, accumulate oxalate in the range of 3-80% (w/w) of their dry weight. As much as 90% of the total calcium of a plant can be found in the form of oxalate salt (Nakata, 2003). Raphide (needle-shaped) crystal formation may serve a dual function of calcium regulation and plant defense. The monoclinic monohydrate form of calcium oxalate (whewellite), is the most usual result of biomineralization mechanisms in plants (Jauregui-Zuniga et al., 2003). The whewellite crystallographic symmetry corresponded to the

monoclinic space group P21/c with cell parameters: $a = 6.290 \text{ \AA}$, $b = 14.580 \text{ \AA}$ and $c = 10.116 \text{ \AA}$, $\alpha = 90$, $\beta = 109.46$, and $\gamma = 90$. Then, comparing the pattern of *B. antiacantha* fruits with that of whewellite (reported in the JCPDS 75-1313 card), one can see a reasonable agreement between the most intense diffraction lines.

Many plants that belong to edible families often contain oxalates. However, there are few reports of any of them causing problems. A few rare cases have been reported, mainly in animals (Bhandari and Kawabata, 2005; Chen et al., 2002). It is possible that the calcium oxalate crystals are partially responsible for the expectorant mechanism of action, which supports the use of these fruits to treat coughs, together with the presence of acid sugars, which may play a part in the mechanism of action as demulcents, protecting against inflammation (Ziment, 2002).

The dry fruits without seeds, ground, were analyzed to determine their composition: 45% carbohydrates, 18% lipids and 1.4% ashes (Table 1). The total lipids fraction (18.2%) can be considered high, since sunflower or soy seeds present 22.1 and 25.9% of lipids in its composition. The fatty acids composition (Table 1) of the lipid fraction presented mainly palmitic (30.12%), linoleic (30.7%) and oleic acids (20.1%), being 25.3% monounsaturated, 39% polyunsaturated and 35.7% saturated. High levels of saturated acids can be considered when comparing to other vegetal oil such as cotton, corn, soy and sunflower, with 25.5; 15.85; 16.4 and 10.85% respectively. The monounsaturated concentration is similar to other species, and the polyunsaturated is lower than the same species cited above: 53.05; 48.8; 59.3 and 65.3%, respectively (Anvisa, 2005).

As described previously, the defatted fraction of *B. antiacantha* was exhaustively extracted generating three fractions: The water-soluble polysaccharides (PS), the NaOH/Borate 50 °C soluble polysaccharides (HT), and not soluble in both solvents (LC) (Table 2).

Analyzing the results obtained, it can be observed that there was no significant difference between the quantities of carbohydrates present in the samples of soluble polysaccharides in water and lignocelluloses, but there was a large difference between these samples for the total hemicelluloses. This difference may be explained by the fact that the sample of total hemicelluloses was not solubilized in the expected manner, preventing total efficiency of the method used to determine its carbohydrate content. The quantity of proteins did not present a significant difference between the samples. Also the moisture content of the samples did not show a significant difference between the samples of water-soluble polysaccharides and total hemicelluloses, however, there was a significant difference between the lignocelluloses sample and the other samples.

Table 3 indicates probably an acid arabinoxyylan as main component of the polysaccharides water-soluble

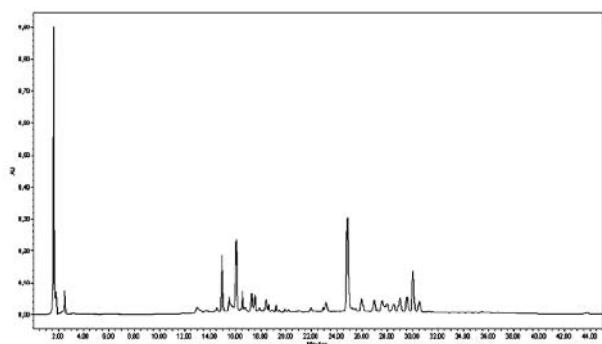


Figure 1. Chromatogram of the crude extract of *B. antiacantha* (330 nm). Gradient system comprised of water acidified with 0.05% acetic acid (A) and methanol (B) 40 minutes - 100% of A up to 20%, flow 0.8 mL/min.

fraction, also known as heteroxylans, substituted by arabinose and glucuronic acid. The total hemicelluloses fraction indicated galactoxyloglucan, arabinogalactan and/or manans, besides acid molecules obtained. The lignocelluloses fraction could be observed high levels of glucose and xilose. In this fraction could be observed

cellulose and arabinoxilans with low solubility, and possibly acetylated.

Through HPLC, it was possible to observe the presence of some phenolic compounds, but none of the standards of flavonoids monitored were found (rutin, quercetin, hesperidin, vitexin, isovitexin, orientin, chrisin, hyperoside). Table 4 shows all the peaks obtained from the above flow.

The total phenolics found for the extracts tested (lyophilized, methanol and lipid) provide absorbency at a concentration of 10 mg/ml, giving the following average values: 12.44, 13.56 and 31.33 $\mu\text{g/ml}$. Every 10 mg (equivalent to approximately 30 mg of ripe, hydrated fruit) of aqueous or methanol extract contains an average of 15 μg of phenolic compounds, or 1500 $\mu\text{g/g}$ (1.5 mg/g) of fresh fruit, which is similar to the values reported for pineapple (1.3) and less than that of mango (2.4) and tamarind (3.9), among others (Soong and Barlow, 2004). This content found may not be directly proportional to the antioxidant capacity or capacity to capture free radicals, depending on the quality of phenolics found.

The lyophilized aqueous and methanol extracts

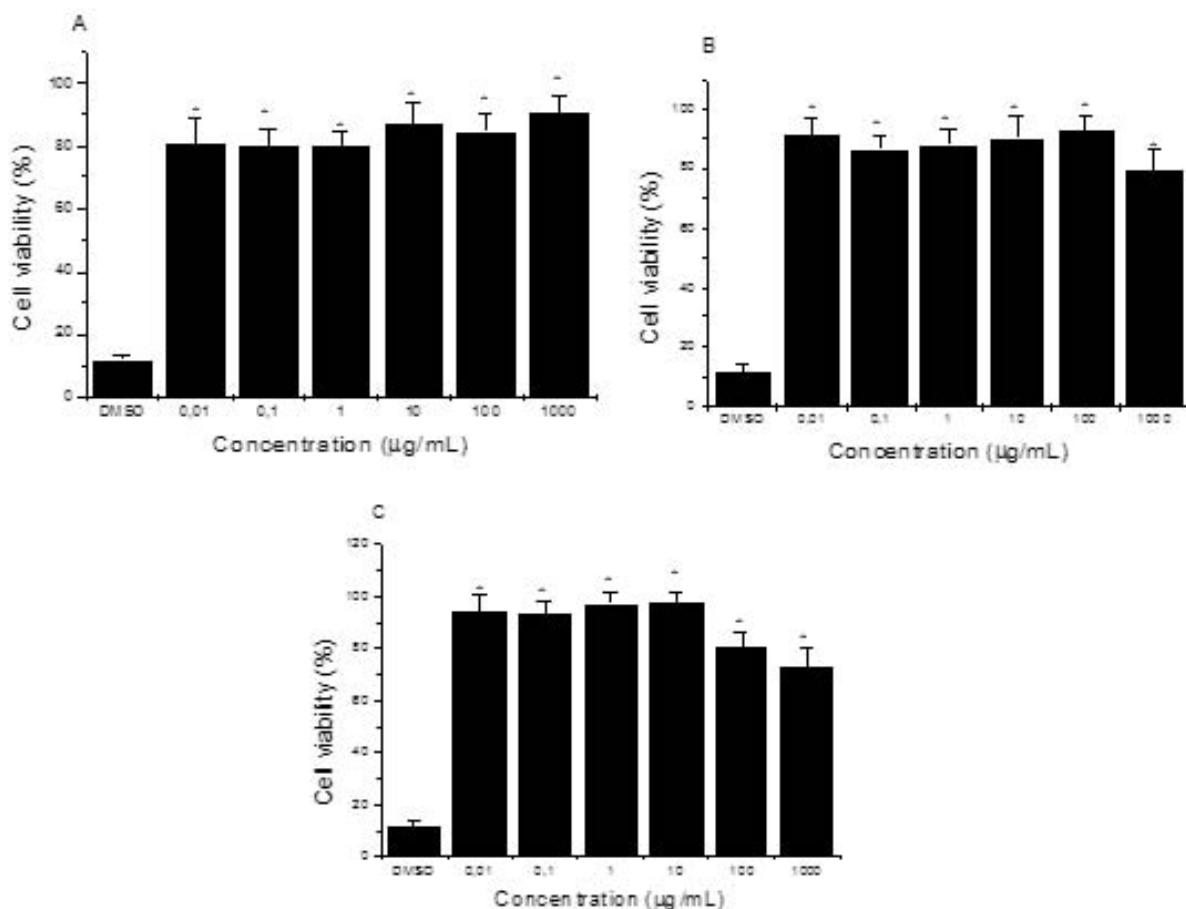


Figure 2. Methanol (A), aqueous (B) and lipid (C) extract of *B. antiacantha* effect upon cell viability as a function of concentration (from 0.01 to 1000 g/mL). Each extract was incubated with cell culture medium for 48 h and cell viability was measured by MTT assay (n=8). DMSO was used as positive control.

Table 1. Chemical composition of the dry, ground fruit without seeds and composition of fatty acids in lipid extracts of dry fruit of *B. antiacantha*.

	% (m/m)	Composition		Percentage
Carbohydrates	44.8			
Proteins	8.3			
Lipids	18.2	Palmitic	C 16:0	30.12
		Palmitoleic	C 16:1	5.25
		Stearic	C 18:0	2.37
		Oleic	C 18:1n 9 _c	20.1
		Linoleic	C 18:2n 6 _c	30.67
		Linolenic	C 18:3n 3	8.28
		Ligniceric	C 24:0	3.18
Moisture	27.3			
Ashes	1.4			

Table 2. Chemical composition of subfractions after lipid extraction of the dry fruit.

Samples	Carbohydrates ^a	Proteins ^b	Moisture ^c
PS	44.87	5.97	35.68
HT	18.46	6.99	33.49
LC	48.43	6.92	11.32

a Method of DUBOIS et al. (1956); PS: Water-soluble polysaccharides

b Method of BRADFORD (1976); HT: Total Hemicelluloses

c AOAC method; LC: Lignocelluloses.

Table 3. Monosaccharide composition of the subfractions after lipid extraction of the dry fruit.

Samples	Monosaccharides (mol %)						
	Glc	Xyl	Gal	Man	Ara	Fuc	AA*
PS	6.8	62.5	7.3	1.9	21.5	0.4	27.8
HT	32.6	22.4	9.2	20.5	15.6	-	17.2
LC	36.8	43.3	1.5	3.1	15.8	-	20.5

* Acid sugars (Blumenkrantz & Abboe Hansen, 1973).

PS Water-soluble polysaccharides

HT Total Hemicelluloses

LC Lignocelluloses.

Table 4. Analysis of HPLC data for the *B. antiacantha* extract.

Retention time (min)	λ max (nm)	Phenolic class
14.97	243, 326	Unknown
15.50	245, 310	Unknown
16.12	226, 312	Unknown
16.56	220, 325	Unknown
17.31	288, 326	Unknown
17.54	239, 329	Unknown
18.42	220, 326	Unknown
19.26	226, 308	Unknown
23.20	255, 341	Flavone derivative
24.84	264, 344	Flavone derivative
26.00	327	Hydroxycinnamic acid
26.97	226, 264, 328	Flavone derivative
27.54	227, 264, 327	Flavone derivative
28.03	231, 264, 327	Flavone derivative
28.52	233, 327.3	Hydroxycinnamic acid
29.05	233, 327.3	Hydroxycinnamic acid
29.63	233, 326	Hydroxycinnamic acid
29.98	236, 328	Hydroxycinnamic acid
30.60	233, 326	Hydroxycinnamic acid

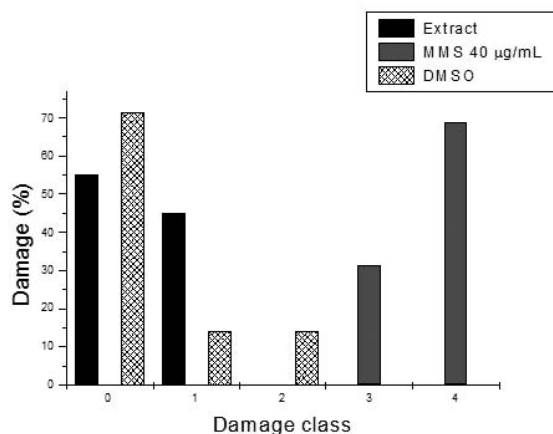


Figure 3. Comet assay on marrow cells obtained of mouse femur. The figure shows the % of damage as a function of damaged class.

using DPPH and complex phosphomolybdenum were tested. Low antioxidant activity has been observed in all the extracts, with the lyophilized aqueous and methanol extracts presenting a similar profile in relation to lack of activity, tested up to 5 mg/ml of extract.

Tests were carried out on the viscosity of the water-soluble polysaccharide sub fraction, at a concentration of 5 g/L, and it can be concluded that the viscosity of the sub fraction is similar to that of water (1 mPa.s), with a Newtonian behavior.

The fibroblasts cytotoxicity of methanol, aqueous and lipid extracts were evaluated at concentrations of 0.01, 0.1, 1, 10, 100 and 1000 µg/mL, as showed in the figure 2.

At 1, 24 (data not shown) and 48 hours (Figure 2), all extract and fractions showed practically absence of cytotoxicity. Only in methanol extract (48 hours) was possible to observe a viability reduction of

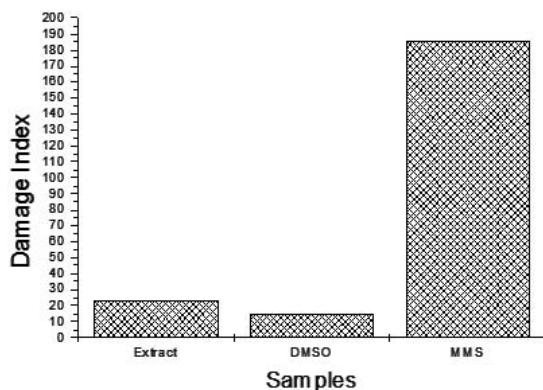


Figure 4. Comet assay on marrow cells obtained of mouse femur. The figure shows the total damage index for Extract (1000 µg/mL); DMSO and MMS (40 µg/mL).

approximately 20%, with any statistical significance observed among the samples concentration, from 0.01 to 1000 µg/mL in 1, 24 or 48 h.

Interestingly, within 1 and 24 hours a slight increase in the viability was observed. The possibility of cell grown was discarded due the absence of calf serum in the media during the execution of the test. So, to confirm if the fractions methanol, aqueous or lipid induced the cell growing at 1 and 24 hour, was carried out another endpoint for the cytotoxicity test, using vital stain neutral red (data not shown) instead of MTT. By neutral red viability endpoint, any statistical significance was observed between the samples and the positive control, indicating that the increase in cell viability using MTT stain was probably due a transient effect of the fractions on the mitochondrial enzyme succinate dehydrogenase (enzyme reducing MTT).

The genotoxicity assay carried out for the wild banana extract, is presented in the Figures 3 and 4.

In the Figure 3, lower damage classes (0 and 1) were observed for wild banana extract at 1000 µg/mL. In Figure 4 is possible to assess that any statistical significant genotoxicity was observed for the extract when compared with the negative control (DMSO). Also, a lower genotoxicity of extract could be observed when it is compared with the positive control (MMS).

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