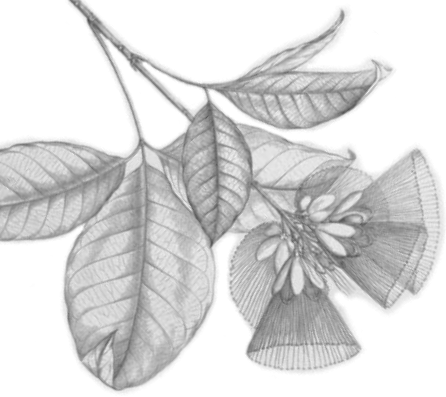

Gum of *Terminalia argentea* Mart. Et. Zucc. (Combretaceae) as a Resource in Biotechnology Processes: Chemistry and Toxicity

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ABSTRACT

Biodegradable polymers are in areas such as pharmaceutical, food and bioremediation processes. Isolation, characterization (SEM, FTIR, TGA, Monosaccharide Composition, Zeta Potential), antimicrobial activity and toxicity to *Artemia salina* and *Allium cepa* L. from the *in natura* gummy exudate of *Terminalia argentea* (GTa) are presented herein. The data show irregular morphology and depressions, bands in 1770, 2112, 1354 cm^{-1}, indicating groups of polysaccharides, amphiphilic character, thermo-degradation above 300 ° C and low water activity (0.6). The assays were negative for *S. aureus* and *E. coli*, nontoxic against the bioindicators tested. GTa presented low cost, extracted and isolated in a sustainable way, with possibility of commercial exploitation by communities, since these biopolymers present possibilities of applications as agents in the support of immobilization of enzymes, as sensors in diagnostics, encapsulating material in processes of microencapsulation of drugs and additives in food industries.

Keywords: Capitão-do-Campo; Biopolymer; Ecological Toxicity.

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Terminalia argentea Mart. et Zucc. (Combretaceae), popularly known as "amêndoa-brava", "cerne-amarelo", "capitão-do-campo", "catinga-de-porco", "mussambé", is a medium size tree found in the Brazilian middle-west region (Garcez et al. 2003; Ribeiro et al. 2017). *T. argentea* adapts very well to poor soils, and it is used in programmes to recover degraded areas and, for its exuberant beauty, in urban gardens. In the construction sector, its wood is used due to its hardness and resistance, and the ash obtained from its burning is useful in the tanning of leather and preparation of soap (Lorenzi 2008).

The family Combretaceae is constituted of nearly 600 species, with two genera of greater occurrence: *Combretum* and *Terminalia*, each with nearly 250 species (Saleem et al. 2002) with antifungal, antimicrobial, antioxidant, antidiabetic, anti-HIV and antimalarial properties in studies with leaves and bark (Rao et al. 2003). Ribeiro et al. (2017) reported that riverine communities living in the North Araguaia microregion of Mato Grosso, Brazil using medicinal infusions made with *T. argentea* leaves for treating gastric ulcers, bronchitis and haemorrhages; the macerated bark for treating ulcers and flu with fever; the bark decoction for diarrhoea, inflammation, wounds, cramps, cancer and as a tranquiliser and diuretic; the flower infusion for anxiety and finally the macerated roots for rheumatism and body pains.

Plants of genus *Terminalia* (Figure 1) are widely distributed in tropical areas worldwide and are known as a rich source of secondary metabolites, such as pentacyclic triterpenoids and their glycosylated derivatives, flavonoids, tannins and other aromatic composts (Garcez et al. 2003; Araújo & Chaves 2005) and the production of gummy exudate (gum) is also a characteristic of this species (BeMiller & Huber 2010; Andrade 2013). The term gum refers to exuded polysaccharide hydrocolloids (Prapajati et al. 2013) which have a wide variety of compositions and rheological properties that can not be easily mimicked by synthetic polymers; are compounds of multiple sugar units linked to each other to create large molecules with heterogeneous composition that, after hydrolysis, produce simple sugar units such as arabinose, galactose, glucose, mannose, xylose or uronic acids (Albuquerque et al. 2016).

Polymers extracted from plants are polysaccharides originating from various parts of the plant such as cell walls, tree exudates, seeds, tubers and roots (Mirhosseini et al. 2008). It can be observed in all parts of the tree and its composition (qualitative and quantitative) depends on the maturity of the trees and the environmental conditions (De Paula et al. 1998; Ribeiro et al. 2016). These properties have several advantages such as solutions in solids with low viscosity, low cost of obtaining, low risk of side effects, biocompatibility, ecological processing, biodegradability, besides the availability in a sustainable way.

Figure 1. Gum *in natura* of *Terminalia argentea*. The gum *in natura* (GTa) was obtained through incisions (10 cm long and 2 cm deep) made in triplicate in each branch of 10 species of *Terminalia argentea* planted in the municipality of Hidrolândia (814 meters of altitude, 16° 99' 11.63" S; 49° 83' 22.10" W), in February 2016, with a monthly average temperature of 26 °C and a monthly average precipitation of 13 mm according to the Meteorological Station (2016). The botanical identification was made by Prof. Dr. Eli Regina Barboza e Souza, and the exsiccates of the species deposited in the Herbarium at the Universidade Estadual de Goiás with the number 11.385. Exudate nodules from the incisions were shredded and stored in hermetically sealed vials at 4 °C until use.

Source: Authors.

The gum produced has colloidal proprieties, thickening, gelling agents, emulsifiers, stabilizers, binders (Marques & Xavier-Filho 1991) and biodegradable functions. Polymeric systems obtained by mixing and/or in combination with different polymers in the form of gel, particles, microcapsules, immobilized complexes and construction of nets and films have received attention of researchers in recent years mainly due to their use in the pharmaceutical, medical (Yamashita & Fernandes 2012). In addition, it is important to note that there is a lack of knowledge about the nature of the product.

Several exudate present industrial applications, such as the arabic gum (*Acacia senegal* L. Willd.), tragacanth gum (*Astragalus gummifer* Labill), karaya gum (*Sterculia urens* Roxb.), cashew gum (*Anacardium*

occidentale L.) (Nussinovitch 1997) and the Cerrado-arboreal cashew gum (*Anacardium othonianum* Rizz) (Silva et al. 2017). There is a growing demand for the use of natural compounds, both in the field of supplements (pharmaceuticals and food) and in their application to industrial processes, since sustainable materials open new technologies and perspectives in the field of science, having as their morphological and (Zuin et al. 2017). In terms of the use of carbohydrates, the size of the productive market in 2015 occurred in more than 1 million tons with a demand outlook of 5 million by 2024 (Gran View Research 2016), being used in active compounds in herbal products, nutraceuticals and food (Zuin et al. 2017) as well as other biotechnological bases. With efficient, green, selective and high value extractions and purifications, cardohydrates are a considerable challenge for the development of economically viable resources for their use and growing market (Gran View Research 2016).

Due to the applications of these gums in several industrial areas, the chemical characterization, microbiological and toxicity tests are necessary. In this context, the aim of this study was to isolate, to chemically characterize (through tests performed by scanning electron microscopy, infrared and thermogravimetric analysis, water activity, zeta potential assessment, protein content and the presence of phenolic compounds, as well as the identification of monosaccharides), to evaluate the microbiological potential (in strains of *Staphylococcus aureus* (ATCC 29213) and *Escherichia coli* (ATCC 25312)) and the toxicity (in *Artemia salina* and *Allium cepa*) of the gum *in natura* of *Terminalia argentea* (GTa), promoting this study as the pioneer in this area, reflecting the importance of studies and applications with Cerrado species, specifically those with potential of these gum production.

MATERIALS AND METHODS

Butyl alcohol p.a., methyl alcohol p.a., monobasic sodium phosphate, potassium dichromate, phosphoric acid, hydrochloric acid, sulfuric acid and vanillin were obtained from Sigma-Aldrich (USA). Giemsa stain was obtained from Merck. Sodium chloride as well as the sugars glucose, sucrose, mannose, galactose were used as standards for chromatography and diphenylamine were obtained from Vetec/Sigma-Aldrich. Thin-layer Chromatography (TLC) plates were obtained from Alugram® Sil G/UV (Germany). For toxicity tests in *Artemia salina*, we used eggs/cysts of Maramar PET.

Staphylococcus aureus (ATCC 29213) and Gram-negative *Escherichia coli* (ATCC 25312) standard strains from the collection of the microbiology laboratory at the Universidade Estadual de Goiás were used for the minimum inhibitory concentration tests. The nutrient broth and agar used in the preparations were Mueller Hinton (Himedia®) and BHI (Brain Heart Infusion) (Acumedia®). The resazurin dye was purchased from Vetec.

OBTAINING AND CHARACTERIZATION

The gum *in natura* (GTa) was obtained through incisions (10 cm long and 2 cm deep) made in triplicate in each branch of 10 species of *Terminalia argentea* planted in the municipality of Hidrolândia (814 meters of altitude, 16° 99' 11.63" S; 49° 83' 22.10" W), in February 2016, with a monthly average temperature of 26 °C and a monthly average precipitation of 13 mm according to the Meteorological Station (2016). The botanical identification was made by Prof. Dr. Eli Regina Barboza e Souza, and the exsiccates of the species deposited in the Herbarium at the Universidade Estadual de Goiás with the number 11.385. Exudate nodules from the incisions were shredded and stored in hermetically sealed vials at 4 °C until use.

The morphological analysis of the GTa was performed by scanning electron microscopy (Shimadzu, model SSx 550, Japan), with 50x -1000x magnification.

GTa samples were analyzed by Fourier-transform infrared spectrometry (FTIR) with KBr pellets (Bomn FT-IR model MB100, USA) scanned in the range of 4000 to 500 cm^{-1}. The spectrum were compared to identify the functional groups present in the samples, according to Silverstein et al. (2013).

The thermal stability of the GTa was evaluated by thermogravimetric analysis (TGA), using the methodology of Lomonaco et al. (2012). The samples were subjected to heating ramps of 25 ^{o}C to 500 ^{o}C at the rate of 3 ^{o}C min^{-1} using DTG-60H (Shimadzu, China).

The molar absorption of the GTa samples (1mg mL^{-1}, p/v) dissolved in distilled water, 0.1 mol L^{-1} sodium chloride and 0.1 mol L^{-1} pH 7.0 monobasic sodium phosphate buffer was performed in a Kasuaki U/VIS spectrophotometer model IL-592 (Japan), in the bands of 280 to 800 nm, with intervals of 20 nm.

The surface charge density of the GTa was determined using the Zeta potential meter (ζ), using a microelectrophoresis measuring chamber (ZetaSizer Nano-ZS, Malvern Instruments Ltda., Worcestershire, UK), carried out at 25 °C in the samples diluted in milliQ water (pH = 7.0).

The pH of the GTa solution (1mg mL^{-1}, p/v) was determined after homogenization using the pHTek digital pH meter, according to the Agência Nacional de Vigilância Sanitária (ANVISA) (Brasil 2010).

The water activity of the comminuted sample of the GTa was measured in the AquaLab (Pre-Water Activity Analyzer) apparatus, in which 7 g of the dry sample were weighed, transferred to the analyzer flask of the apparatus, and analyzed in direct reading using an electrolytic sensor.

The moisture content of the GTa was determined according to the method described in the Farmacopeia Brasileira (Brasil 2010), in which 1g of the sample was weighed, ground and placed in an oven at 105 °C for 24 hours, until constant weight was obtained. The moisture content was calculated by the difference between the initial and final weights of the samples, the final value being expressed as a percentage (%) according to equation (1):

$$\%\text{moisture} = \frac{m_{as}}{m_{au}} \times 100\% \quad (1)$$

being m_{as} referring to the value of dry mass (g) and m_{au} to the value of wet mass (g).

The presence of soluble proteins in GTa samples was investigated by the method of Bradford (1976), using bovine serum albumin and spectrophotometric reading at 595 nm as standard (Kazuaki, Japan).

The identification of simple phenolic compounds, flavonoids and anthraquinones was performed in GTa according to Radi &Terrones (2007) methodology. In 8g of GTa were added 80 mL of 70% (v/v) ethanol. The material was boiled for 5 min and filtered on filter paper moistened with 70% (v/v) ethanol. This material, identified as an extract, was used for the reactions for characterization.

Preliminary identification of the carbohydrate composition was performed by thin-layer chromatography (TLC) using 10 mg mL^{-1} of GTa dissolved in 6% (v/v) hydrochloric acid (HCl) and shaken for 2 h, followed by autoclaving for 1h. 30 µL of the solutions of: 10 mg mL^{-1} GTa dissolved in 6% HCl, 5 mg mL^{-1} of carbohydrate markers (glucose, sucrose, mannose and galactose) (Chung et al. 1995) were applied. The TLC was conducted according to Chung et al. (1995) using as the mobile phase a solution of n-butanol / methanol / water (4: 2: 1). After running, the plates were oven dried at 70 °C and developed with sulfuric vanillin solution. Strip development was done after incubation of the plates in oven at 90 °C for about 30 min.

The determination of neutral monosaccharides was performed by Wolfrom & Thompson (1963). The polysaccharides were hydrolyzed with 2 M trifluoroacetic acid for 2 hours at 120 °C. After evaporation of the acid, the monosaccharides were reduced, acetylated and the alditol acetates analyzed by liquid-gas chromatography using Trace GC Ultra (Thermo Electron Corporation) equipment and

column capillary DB-225 (0.25 mm x 30 m). The injector and flame ionization detector temperatures were 250 °C and 300 °C, respectively. The oven temperature was programmed from 100 °C to 215 °C at a heating rate of 40 °C / min. The entrainment gas was helium in flow rate of 1.0 mL / min.

MICROBIOLOGICAL TESTING

The tests to determine the minimum inhibitory concentration were performed based on the protocol of CLSI (2010) to determine the sensitivity to antimicrobial agents for aerobic growth bacteria.

Each strain of *Staphylococcus aureus* (ATCC 29213) and *Escherichia coli* (ATCC 25312) was seeded on BHI agar and incubated at 36 °C for 24 hours in culture oven. After this period, 3 to 5 isolated colonies were transferred to a tube containing 5 mL of 0.9% saline solution and the turbidity corresponding to 0.5 of the McFarland scale (10^8 CFU mL^{-1}) was checked. The solution was then diluted 1:10 in physiological solution (1 mL of microorganism suspension in 9 mL of 0.9% saline solution) to give a concentration of 10^7 UFC mL^{-1}. The procedure of diluting the suspension was performed 15 minutes prior to inoculation of the strains into the wells of MIC microplates. All materials and support instruments for the experiment were autoclaved and performed in a laminar flow chamber.

The concentrations of GTa analyzed were 3.12; 6.25; 12.5; 25; 50 and 100 mg mL^{-1}, under conditions similar to that used by Araújo et al. (2015) for *A. occidentale* extract. For each concentration of the test compounds, 100 µL was pipetted and placed into the wells labeled for the 96-well microplates intended for testing. A line of wells was destined to the test solutions of each concentration, without inoculum for control of medium and substance. A column with 100 µL of medium and inoculum (without test substance or solvent) was destined to control the viability of the strains.

Once the plate was filled, 5 µL of each inoculum was pipetted into the labeled wells. The 96-well microplates were then capped and incubated at 36 °C for 22 hours. Subsequently, 20 µL of 0.1% resazurin solution in 0.9% sterile saline solution was added and incubated for an additional 2 hours.

The reading was performed in spectrophotometer at 570 nm and 600 nm according to manufacturer resazurin for calculating the percentage of bacterial growth inhibition. Visually, the pink color is considered as proof of bacterial growth and the blue color indicates no proliferation of microorganisms. All tests were performed in triplicates.

The test for antimicrobial activity was performed using the disk diffusion method based on the CLSI (2010) protocol to determine the sensitivity to antimicrobial agents for bacteria of aerobic growth. For the test, the bacterial strains were inoculated on Mueller-Hinton agar using a sterile swab, distributing the inoculum uniformly on the surface of the agar. The previously sterilized filter paper disks (5 mm diameter) were placed in the plate and embedded with 3 µl of the concentrations (3.12, 6.25, 12.5, 25.50 and 100 mg mL^{-1}) of the GTa. Discs used as a positive control were prepared at a concentration of 30 µg chloramphenicol. Subsequently, the plates were sealed and incubated at 36 °C for 24 hours. After the growth period the evaluations were performed by analyzing the inhibition zone diameter.

TOXICITY TEST

For the assays in *Allium* cepa L., the samples were commercially obtained in the municipality of Anápolis, State of Goiás, Brazil. The experimental groups were composed of GTa (10 mg mL^{-1}), crude ETE (the effluent samples were collected at the Estação de Tratamento de Efluentes (ETE) of the Distrito Agroindustrial de Anápolis (DAIA) (16°30'S and 49°00'W). The organic load after the collection of 24 hours in the raw sewage, according to the Department of Environment of the DAIA, is 3,750 kg $BOD.dia^{-1}$) in addition to the negative (distilled water) and positive (Azide Sodium 0.02gL^{-1}). Ten bulbs were distributed for each material to be tested, placed in glass containers with root area directly in contact with water and exposed for 48 hours. The bulbs were measured and transferred to containers containing the test solutions for 72 hours and then again measured and used for cytogenetic analysis (the toxic potential was measured by root length analysis (in centimeters)). The roots were fixed in Carnoy's solution (99% ethanol, 3:1 glacial acetic acid). For the hydrolysis, a 5.0 mol L^{-1} HCl solution was applied to the material of smear preparation; the slides were dipped in liquid nitrogen for 30 seconds and then stained with 10% (p/v) Giemsa. The mitotic index (MI) and the frequency of mitotic cycle abnormalities (MCA) were evaluated in the slide's analysis. The MI was determined using equation (2):

$$IM = NCM \div NTC \times 100 \quad (2)$$

where NCM corresponds to the number of cells in mitotic division and NTC to the total number of cells analyzed (Pereira 2015).

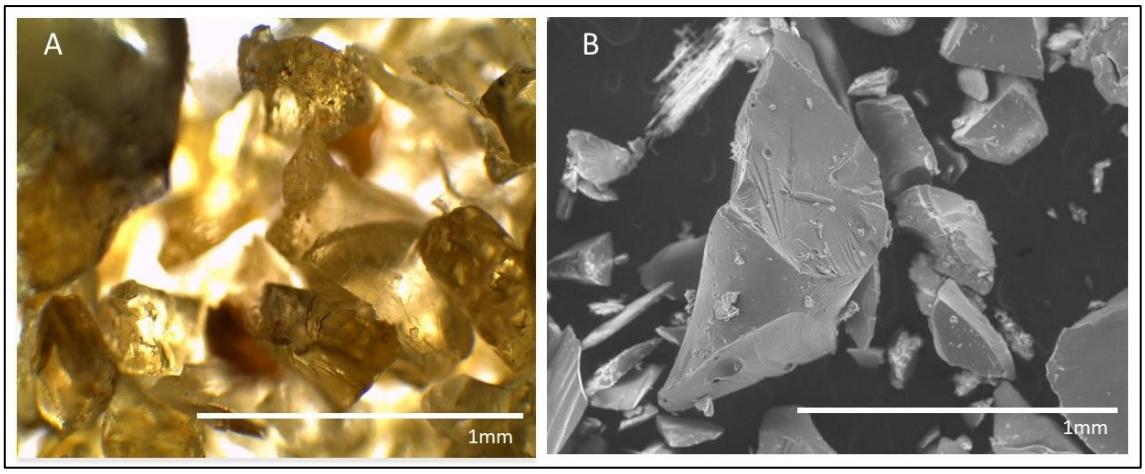
The bioassay with *Artemia salina* was performed on a 96-well polystyrene microplate with concentrations of GTa in 3.12; 6.25; 12.5; 25; 50 and 100 mg mL^{-1}, respectively, according to Molina-Salinas & Said-Fernández (2006) methodology. For the cysts eclosion, it was used the synthetic marine

water medium prepared with the dissolution of sea salt in 36% (p/v) distilled water supplemented with yeast extract (6.0 g L^{-1}) and sterilized by autoclaving. The cysts were incubated for 36 hours in the medium with natural light at 26 °C and constant oxygenation. After hatching, the nauplii were transferred to a petri dish and, using a micropipette 10 larvae were distributed to each microplate well in the presence of the gummy exudate *in natura* at different concentrations. After 24 hours of incubation, counts of live and dead individuals were performed. Negative control (saline solution) and lethality (potassium dichromate at 12.5 µg mL^{-1}) were included in the tests. To obtain the LC_{50} values, the PROBIT analysis was used through the STATISTICA® software. Compounds whose LC_{50} were less than 1000 µg were considered to be toxic; within this range of 0-1000 µg concentrations of 250-1000 µg were considered to be of low toxicity; medium toxicity from 250-80 µg and high toxicity concentrations lower than 80 µg (Dolabela 1997).

RESULTS AND DISCUSSION

Scanning electron microscopy for the GTa (Figure 2) showed an irregular and crystalline appearance, with presence of depressions and presence of relief, similar to the morphological aspect of *Anacardium occidentale* polysaccharide found by Ramalho (2014) and of the Cerrado arboreal polysaccharide (*Anacardium othonianum* Rizz) found by Silva et al. (2017). These crystalline and amorphous forms in polymeric materials may show differences in particle size and shape, physicochemical properties, chemical stability, water solubility and hygroscopicity (Cano-Chauca et al. 2005).

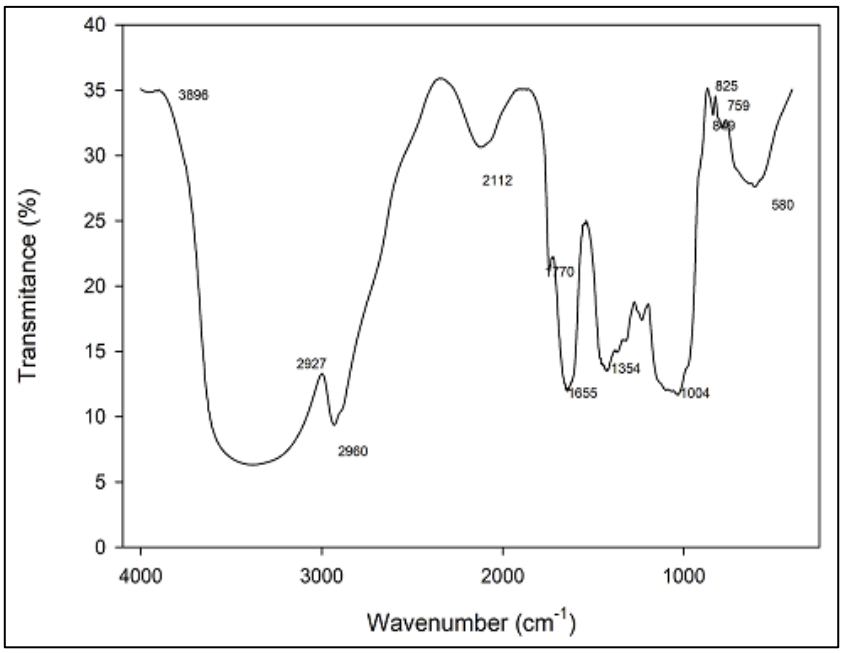
Figure 2. Optical microscopy (A) and electromyrograph (B) of the gummy exudate of Terminalia argentea. Increase by 100x in (A) and (B), respectively.



Source: Authors.

The infrared spectrum of the GTa (Figure 3) presented polysaccharide bands, such as those presented in band of 3896 cm^{-1} (OH stretching). The band in the 2927 and 2960 cm^{-1} region show C-H bond, composing the polysaccharide throughout its structure. Very strong bands which occur at 2854-3008cm^{-1} in the gums can be mostly attributed to absorption of CH stretch and CH_2- asymmetric stretch in aliphatic groups closer to those bands (Arthur et al. 2014). C-O stretch vibration due to carboxylic acid was noticed at 1354 cm^{-1}. The (C-C) vibrational mode appeared at 1655 cm^{-1}. Study with gum arabic (*Acacia senegal*) performed by Daoub et al. (2016) showed bands close to 2926 cm^{-1}, indicating for this material the presence of sugars such as galactose, arabinose and rhamnose, since the spectrum shows absorption bands characteristic of glycosidic bonds, 816 cm^{-1} υ C-O attributed to glycosidic bond α-D-galactopyranose and in 873 cm^{-1} υ C-O glycosidic bond of β-D-mannopyranose type. The bands present in the range of 1870 to 1540 cm^{-1}, which are related to the presence of groups - C = O, (Sekkal et al. 2003) are worth mentioning. The region between 750-950 cm^{-1} could be credited to various types of vibration of the piranosidic rings and glycosidic bonds.

Figure 3. Infrared spectrum of the gummy exudate of *Terminalia argentea*. Samples were analyzed with KBr pellets (Bomn FT-IR model MB100, USA) and swept in the range of 4000 to 500 cm^{-1}.

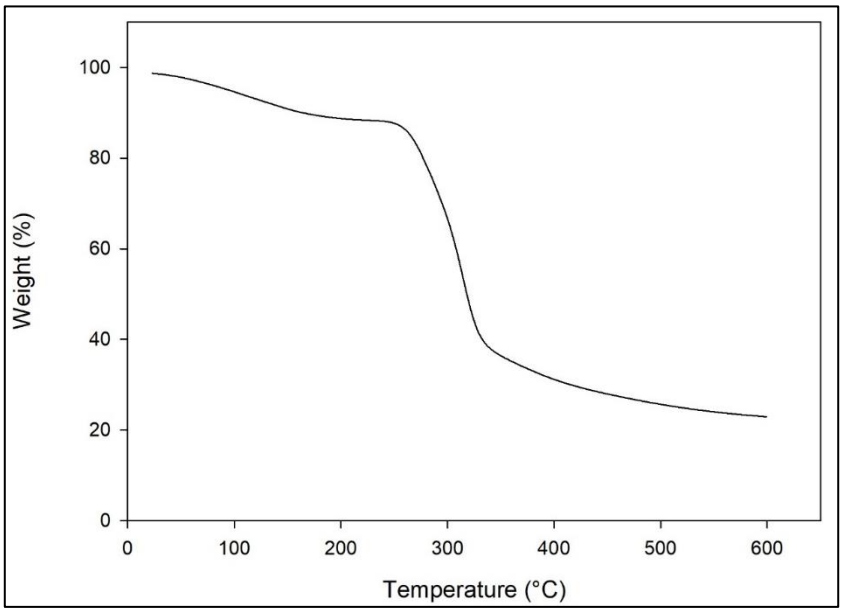


Source: Authors.

Figure 4 shows the thermal analysis of GTa. It is noticeable the decomposition in two stages: the first around 265 °C and the second around 336 °C, which highlights the importance of this species in the production of this gummy exudate, due to the potential of its application in several industrial sectors due to stability in high temperatures, as for example in the production of microcapsules by the

spray drying method, using these polymeric materials as a wall material. The use of the *spray dryer* is justified because it is the drying method for microencapsulation with less cost when compared to the other methods and, although the technique uses high temperatures, the rapid evaporation keeps the droplet temperature low, making the technique possible for sensitive products to the heat, as biological and pharmaceutical products, without affecting the final quality of the product (Alves et al. 2014).

Figure 4. Thermogravimetric analysis of gummy exudate of *Terminalia argentea*. The samples were subjected to heating ramps of 25 ºC to 500 ºC at the rate of 3ºC min $^{-1}$. using DTG-60H (Shimadzu, China).



Source: Authors.

Studies with gum arabic showed the maximum degradation temperature between 270 and 320 °C (Zohuriaan & Shokrolahi 2004) chitosan (300 °C) and guar gum (306 °C) (Varma et al. 1997), xanthones (298 °C) (Villetti et al. 2002). Monthé & Rao (2000) showed for *Anacardium occidentale* only a decomposition stage with maximum temperature around 300 °C and Silva et al. (2017) presented for the Cerrado-arboreal cashew polysaccharide the first decomposition at 320 °C, evidencing the thermal resistance of these two Cerrado gum species.

The pH of the GTa solution in distilled water was 5.46 by enhancing commercially available gum-like chemical properties, such as gum arabic (pH = 5.0), gum karaya (pH = 4.5 to 4.7), gum ghatti (pH = 5.0) and Cerrado species such as the Cerrado-arboreal cashew gum (*Anacardium othonianum*, pH = 5,6) (Silva et al. 2017). It is known that the extraction of polysaccharides in aqueous media, similar to that occurring with cereal β-D-glycans, can be influenced by factors such as pH, temperature and time (Ramesh & Tharanathan 1999).

The presence of flavonoids, chalcones, and isoflavones in the GTa is described in Table 1.

Table 1. Summary of results of the presence of phenolic compounds in gummy exudate of *Terminalia argentea.*

Reactions for characterization	Flavonoids	Chalcones	Isoflavones
Cyanidin	-	Positive	Positive
Oxalo-Boric	Positive	-	-
NaOH	Positive	Positive	-
$FeCl_3$	Positive	-	-
$AlCl_3$	Positive	-	-

Source: Authors.
*The legend (-) refers to the absence of these compounds in the sample analyzed. The identification was performed in GTa according to Radi &Terrones (2007) methodology. In 8g of GTa were added 80 mL of 70% (v/v) ethanol. The material was boiled for 5 min and filtered on filter paper moistened with 70% (v/v) ethanol.

The phenolic compounds of plants fall into several categories, such as: simple phenols, phenolic acids (derived from benzoic and cinnamic acids), coumarins, flavonoids, stilbenes, condensed and hydrolyzable tannins, lignans and lignins (Chaves et al. 2010). Species of the genus *Terminalia* are known as a rich source of secondary metabolites, such as tannins (gallic acid and simple gallate esters; chebulic acid; chebulic and non-chebulic ellagitannins; ellagic acid and ellagic acid derivatives and glycosides), flavonoids (quercetin, kaempferol, rutin, luteolin, apigenin, vitexin, isovitexin, catechin, gallocatechin, epicatechin, epigallocatechin and leucocyanidin among others), phenolic acids (caffeic acid, ferulic acid, vanilic acid and coumaric acid among others), triterpenes (ursolic acid, asiatic acid, oleanolic acid, arjunic acid, arjunolic acid, β-sitosterol, stigmasterol and terminalin among others), triterpene glycosides (arjunetin, chebuloside, terminoside, sericoside, bellericoside and daucosterol) and lignans (isoguaiacin, termilignan, thannilignan and anolignan among others) (Fahmy et al. 2015).

Since zeta potential (ζ) is a measurement technique for assessing colloidal stability (Mirhosseini et al. 2008; Porto & Cristianini 2014), the value found for GTa was -34mV. According to Mishra et al. (2009), in order to obtain a physically stable zeta potential, the value must be greater than 30 mV (absolute value); since the aggregation of particles may be present in values between ζ = -5 mV to ζ = + 5 mV. Porto & Cristianini (2014) found in *A. occidentale* gum values of ζ = 1.27mV (in absolute value) while Delfini (2016) had values of ζ = 21.2 mV (in absolute value) for gum arabic. This analysis showed this polysaccharide to be a biopolymer with amphiphilic characteristics, which contains both groups: positively and negatively charged, suggesting a material with potential to be used as an emulsifying and encapsulating agent. The strong hydrophilic characteristic and the favorable rheological property of these polysaccharides are the main reasons for their increasing application as texture modifiers, thickeners, gelling agents and emulsifiers in the food and biomedical industries (Tabarsa et al. 2017). However, the functional properties of the polysaccharides are closely related to their monosaccharide

compositions, molecular weight, chain-chain interaction and glycosidic bonds (Funami et al. 2011). Therefore, research on the physicochemical characteristics and functional properties of polysaccharides may contribute to find potential applications in food and pharmaceutical products.

The water activity, a qualitative measure that makes it possible to evaluate the availability of free water susceptible to various reactions (Labuza 1977), for GTa was $A_w = 0.6$, within the reduced microbiological contamination range (values of water activity less than or equal to $A_w = 0.6$), according to the scale used in food technology. This parameter becomes important in food preservation, both in the biological aspect and in physical transformations, thus evaluating its potential of deterioration (Pitalua et al. 2010).

The moisture content of this polymer material was 27%. This tendency of water adsorption is justified because it is a polysaccharide, a highly hygroscopic molecule (Cano-Chauca et al. 2005), capable of adsorbing water from the environment.

The analysis showed 6.71 g L^{-1} of proteins GTa which, besides the defense role, can act as aggregating agents for the polysaccharide chains, since the protein fraction is co-precipitated with ethanol (Ramesh & Tharanathan 1999). This integrity by the proteins allows the polysaccharide a greater proportion of solubility due to the acquired superficial stability (Prapajati et al. 2013), since the structural integrity starts to present the same susceptibilities that the proteins present.

The preliminary analysis of sugar composition of the GTa was shown by the thin-layer chromatography, with the retention factor (Rf) of 0.91 for the analyzed material and for the other monosaccharides in Glucose (Rf 0.63), sucrose (Rf 0.57), fructose (Rf 0.60), mannose (Rf 0.71), galactose (Rf 0.66); with presence of galactose and mannose, and it is suggested that the material belongs to the polysaccharide group of galactomannans. Similar results were found by Rodrigues et al. (1993) in the analysis of cashew gum and polysaccharide (*Anacardium occidentale* L) and by Silva et al. (2017) with the Cerrado-arboreal cashew polysaccharide (*Anacardium othonianum* Rizz).

Extracts derived from leaves of plants of the genus *Terminalia* analyzed in South Africa showed antibacterial effect, for example the ethanolic extract of *Terminalia chebula* with MIC of 975 μg mL^{-1} against *S. aureus*, explained by the chemical composition of the extract (flavanones, chalcones and flavans, pentacyclic triterpenes and saponins) (Cock & Van Vuuren 2015). *Terminalia sericea* and *Terminalia pruinoides* have been historically used in topic treatment of fungi disturbances, where *T. sericea* (extract of tree bark and leaves) particularly effective, in general with values of MIC of 500 μg mL^{-1} (<5 attached on the disc). However, the evaluation results of the minimal inhibitory concentration (MIC)

and the disk diffusion test at different concentrations of GTa against strains of *Staphylococcus aureus* (ATCC 29213) and *Escherichia coli* (ATCC 25312) did not demonstrate evidence of antimicrobial activity of this material for any concentration analyzed. This data is important in the effective contribution to aspects and applications of degradability of this biopolymer, either through compositions, blends, copolymers, obtainment, separation, general and medical applications, among others. However, it is important to remember that this action depends on factors, such as types of microorganisms, which are more susceptible to enzymatic action. However, it is important to remember that this action depends on factors such as microorganisms (temperature, humidity, pH, light, O_2) and polymer properties (molar mass, crystallinity, etc.) (Franchetti & Marconato 2006).

The bioassay with *Artemia salina* showed that the GTa can be considered to be non-toxic for the microcrustacean, since at the maximum concentration tested (100 mg mL^{-1}) the mortality rate was only 5% and the LC_{50} was estimated at 317 mg mL^{-1}.

Dolabela (1997) attributed a correlation between the degree of toxicity and the median lethal concentration (LC_{50}) where compounds with LC_{50} < 80 μg mL^{-1} are considered highly toxic; between 80 μg mL^{-1} and 250 μg mL^{-1} moderately toxic; LC_{50} > 250 μg mL^{-1} are considered low toxicity or non-toxic and values above 1000 μg mL^{-1} are considered to be non-toxic.

Aqueous extracts of roots and leaves of *Terminalia violaceae* present LC_{50} values of 800 μg mL^{-1} which is considered to be of low toxicity (Cock & Van Vuuren 2015). Parvez et al. (2012) tested a combination of chitosan and gelatine for toxicity evaluation, which was toxic at concentrations of 2.0 mg mL^{-1} to 4.0 mg mL^{-1} due to the material viscosity. Bilal et al. (2016) study with chitosan as support of enzyme immobilization evidenced survival of 63% of this crustacean for this material.

The meristematic cells of the roots of *Allium cepa* L. are suitable indicators for the detection of clastogenic effects caused by environmental pollutants, especially for the monitoring of water and the soil contaminants as well as for the detection of genotoxic effects, monitoring the action of medicinal plant extracts (Camparoto et al. 2002). For the roots of *Allium cepa* L. the results are shown in Table 2.

Table 2. Different treatments in root of *Allium cepa* L. regarding toxicity, cytotoxicity and genotoxicity.

Treatment	Toxicity (%)	Cytotoxicity (%)	Genotoxicity (%)
Distilled water	0	0	0
Sodium azide (0.02 g mL-1)	63.5	25.9	47
GTa (10 mg mL-1)	0	29	6.2
ETE	90	32.4	34

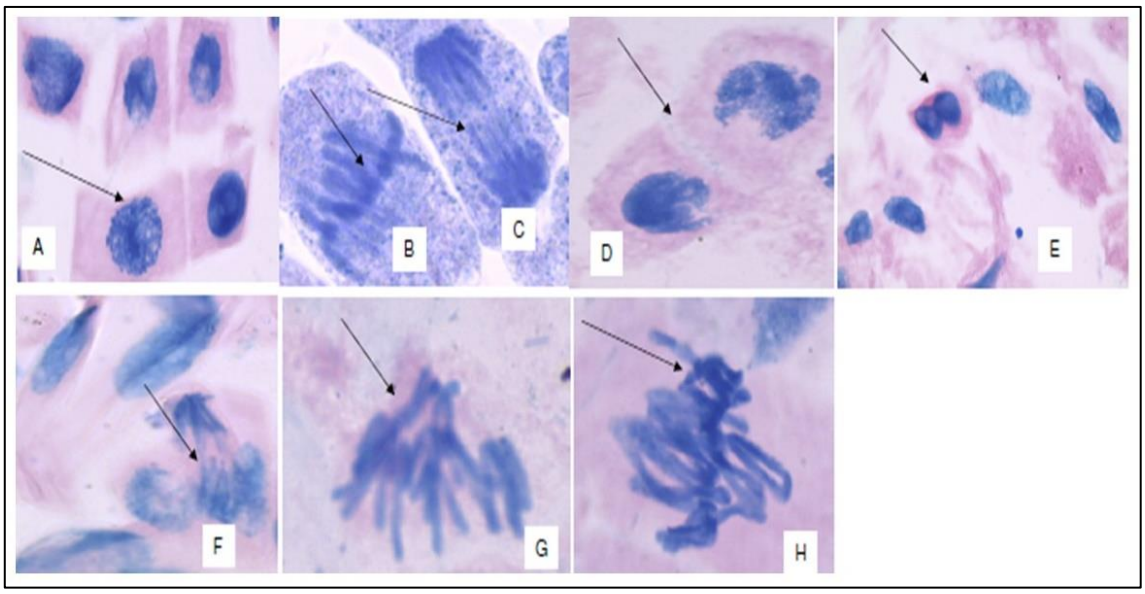
Source: Authors.

Toxic agents should reduce the germination index of *Allium cepa* by more than 50% in relation to the negative control (Fiskesjo 1985). This indicates that the ETE effluent is a toxic material with a reduction effect ($p < 0.05$) higher than the negative control (sodium azide 0.02 g mL^{-1} (63.5%)).

The cytotoxic evaluation is based on the change in the mitotic index (MI) of the different treatments. Based on Table 2, the solutions containing GTa did not promote cytotoxic alterations when compared to the negative control (water) (mitotic index less than 22%).

In the literature, it is considered that cytotoxic interference occurs when the substance is able to inhibit 22% of the mitotic index in relation to the negative control and in a greater amount of inhibition, above 50% (Molina-Salinas & Said-Fernández 2006). We noticed normal mitosis when both negative control and GTa were incubated within the roots of *Allium cepa*. The genotoxicity test aims to identify if the analyzed substance affects cellular vital processes, such as duplication and gene transcription (Luz et al. 2012), being evidenced in Figure 5. Sudhakar et al. (2001) using the *A. cepa* test to evaluate the genotoxic potential of effluents from the silk dye industry reported the inhibition of MI and the induction of several mitotic abnormalities in the roots exposed to these effluents, thus confirming its mutagenic potential.

Figure 5. *Allium cepa* root cells in different treatments: A, B, C, D: Normality of cell division (prophase, metaphase, anaphase, telophase) in water; E: Binucleated cell with *Terminalia argentea* gum solution; F: Bridge, under treatment with sodium azide (0.02 mg mL^{-1}); G, H: Chromosomes lost on treatment with *Terminalia argentea* gum solution.



Source: Authors.

CONCLUSION

The results show the *in natura* gummy exudate of "Capitão do Campo" (*Terminalia argentea*) a promising material as an alternative source for new exuded plant gums. The extraction of the material shows itself in a sustainable way, since there is no death of the plant for the extraction of the material; the solubility in water is high due to the polysaccharide composition (in particular the presence of monosaccharides galactose and mannose); the aggregate protein content is low; this polysaccharide to be a biopolymer with amphiphilic characteristics, suggesting a material with potential to be used as an emulsifying and encapsulating agentt; the absence of toxicity to biomarkers such as *Artemia salina* and *Allium cepa* allows a decrease in the negative impacts of chemical analyzes on the environment and analytical laboratories.

Thus, new materials produced from the gum of this species (associated with other materials or isolates) may attract the interest of the industries involved in the production and commercialization of this product and, thus, increase economic income in other emerging fields, such as silk and pharmaceutical technology through controlled release drug systems.

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REFERENCES

Albuquerque TL, Peirce S, Rueda N, Fernandes-Lafuente R 2016. Ion exchange of β-galactosidase: The effect of the immobilization pH on enzyme stability. *Process Biochem* 51(7):875-880.

Alves SF, Borges LL, Dos Santos TO, Paula JR, Conceição EC, Bara MTF 2014. Microencapsulation of Essential Oil from Fruits of *Pterodon emarginatus* Using Gum Arabic and Maltodextrin as Wall Materials: Composition and Stability. *Dry Technol* 32(1):96-105.

Andrade KCS 2013. Goma de cajueiro (*Anacardium occidentale*): avaliação das modificações químicas e físicas por extrusão termoplástica. *Polimer* 23(5):667-671.

Araújo DV, Chaves MH 2005. Triterpenóides pentacíclicos das folhas de *Terminalia brasiliensis*. *Quim Nova* 28(6):996-999.

Araújo TSL, Costa DS, Sousa NA, Souza LKM, Araújo S, Oliveira AP, Sousa FBM, Silva DA, Barbosa ALR, Leite RSA, Medeiros JVR 2015. Antidiarrheal activity of cashew gum, a complex heteropolysaccharide extracted from exudate of *Anacardium occidentale* L. *J of Ethnoph* 174:299-307.

Arthur DE, Gimba CE, Nnabuk EO 2014. Miscibility Studies of Cashew Gum and Khaya Gum Exudates in Dilute Solution by Viscometry and FTIR Analysis. *AJER* 8(3):01-12.

BeMiller JN, Huber KC 2010. Carboidratos. In S Damodaran, KL Parkin, OR Fennema. *Química de Alimentos de Fennema*. Porto Alegre, p. 75-130.

Bilal M, Asgher M, Iqbal M, Hu H, Zhang X 2016. Chitosan beads immobilized manganese peroxidase catalyticpotential for detoxification and decolorization of textile efluente. *Int J of Biol Macromol* 89:181-189.

Bradford M 1976. A rapid and sensitive method for the quantitaion of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254.

Brasil (Agência Nacional de Vigilância Sanitária) 2010. *Farmacopeia Brasileira* 1(5):.

Camparoto ML, Teixeira RO, Mantovani MS, Vicentini VEO 2002. Effects of *Maytenus ilicifolia Mart.* and *Bauhinia candicans* Benth infusions on onion root-tip and rat bone-marrow cells. *Genet Mol Biol* 25:85-89.

Cano-Chauca M, Stringheta PC, Ramos AM, Vidal J 2005. Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. *Inn Food Sci and Emerg Technol* 6:420-428.

Chaves MA, Citó AMGL, Lopes JAD, Costa DA, Oliveira CA, Costa AF, Brito-Junior FEM 2010. Fenóis totais, atividade antioxidante e constituintes químicos de extratos de *Anacardium occidentale* L., Anacardiaceae. *Rev Bras Farmacogn* 20(1):106-112.

Chung YC, Kobayashi T, Akiba T, Kudo T 1995. Purification and properties of extracellular amylase from the hyperthermophilic archaeon *Thermococcus profundus* DT5432. *Appl Environ Microb* 1(4):1502-1506.

CLSI (Clinical and Laboratory Standard Institute) 2010. Performance Standards for Antimicrobial Susceptibility Testing. 20th Informational Supplement. CLSI document M100-S20. Clinical and Laboratory Standard Institute, Wayne, PA.

Cock IE, Van Vuuren SF 2015. South African food and medicinal plant extracts as potential antimicrobial food agentes. *J Food Sci Technol* 52(11):6879-6899.

Daoub RMA, Elmubarak AH, Misran M, Hassan EA, Osman ME 2016. Characterization and Functional Properties of some Natural *Acacia* Gums. *J Saudi Soc Agri Sci* 17(3):241-249.

De Paula R, Heatley F, Budd PM 1998. Characterization of *Anacardium occidentale* exudate polysaccharide. *Polym Int* 45(1):27-35.

Delfini FT 2016. *Microencapsulação de óleo de semtem de maracujá através da secagem por atomização*, Dissertação de Mestrado em Engenharia de Alimentos, Universidade de Campinas, 113pp.

Dolabela MF 1997. *Triagem in vitro para atividade antitumoral e anti Trypanosoma cruzi de extratos vegetais, produtos naturais e substâncias sintéticas*. Dissertação de Mestrado, Universidade Federal de Minas Gerais, 128pp.

Fahmy NM, Al-Sayed E, Singab AN 2015. Genus *Terminalia*: a phytochemical and biological review. *(Montin.) Species Med Aromat Plants* 4: 218.

Fiskesjo G 1985. The *Allium* test: a standard in environmental monitoring. *Hereditas 102*:99-112.

Franchetti SMM, Marconato JC 2006. Polímeros biodegradáveis - Uma solução parcial para diminuir a quantidade de resíduos plásticos. *Quim Nova* 29(4):811-816.

Funami T, Nakauma M, Ishihara S, Tanaka R, Inoue T, Phillips GO 2011. Structural modifications of sugar beet pectin and the relationship of structure to functionality. *Food Hydrocoll* 25(2):221-229.

Garcez FR, Garcez WS, Martins M, Lopes FA 2003. Triterpenoids, lignan and flavans from *Terminalia argentea* (Combretaceae). *Biochem System and Ecol* 31:229-232.

Gran View Research [homepage on the Internet]. Fibers and specialty carbohydrates market analysis by application (functional food, functional beverage, dietary supplements, animal nutrition, personal care) and segment forecasts to 2024. [updated 2016 Aug; cited 18 set 2018]. *Gran View Research*; [about 3 screens] Available from: https://www.grandviewresearch.com/industry-analysis/fibers-specialty-carbohydrates-market.

Labuza TP 1977. The properties of water in relationship to water binding in foods: A review. *J of Food Process and Preserv* 1:167-190.

Lomonaco D, Maia FJN, Clemente CS, Mota JPF, Costa-Júnior AE, Mazzeto SE 2012. Thermal studies of new biodiesel antioxidants synthesized from a natural occurring phenolic lipid. *Fuel* 97:552-559.

Lorenzi H 2008. *Árvores Brasileiras: Manual de Identificação e Cultivo de Plantas Arbóreas do Brasil,* 5.ed. Instituto Plantarum, Nova Odessa, SP.

Luz AC, Pretti IR, Dutra JCV, Batitucci MCP 2012. Avaliação do potencial citotóxico e genotóxico de *Plantago major* L. em sistemas teste in vivo. *Rev bras Plant med* 14(4):635-642.

Marques MA, Xavier-Filho J 1991.Enzymatic and inhibitory activities of cashew gum exudate. *Phytochem* 30(5):143-1433.

Mirhosseini H, Tan CP, Hamid NAA, Yusof S 2008. Effect of Arabic gum, xanthan gum and orange oil contents on ζ-potential, conductivity, stability, size index and pH of orange beverage emulsion. *Colloids Surf A Physicochem Eng Asp* 315:47-56.

Mishra PR, Al Shaal L, Müller RH, Keck CM 2009. Production and characterization of Hesperetin nano suspensions for dermal delivery. *Int J of Pharm* 371(1):182-189.

Molina-Salinas GM, Said-Fernández S 2006. A modified microplate cytotoxicity assay with brine shrimp larvae (*Artemia salina*). *Pharmacol* 3:633-638.

Monthé CR, Rao MA 2000. Thermal behavior of gum arabic incomparison with cashew gum. *Thermoch Acta* 357:9-13.

Nussinovitch A 1997. Exudate gums. In *Hydrocolloid applications: gum technology in the food and other industries*. Blackie Academic & Professional, London, 345pp.

Parvez S, Rahman MM, Khan MA, Khan MAH, Islanm JMM, Ahmed M, Rahman MF, Ahmed B 2012. Preparation and characterization of artificial skin using chitosan and gelatin composites for potential biomedical application. *Polym Bull* 69(6):715-731.

Pereira AS 2015. *Avaliação do potencial mutagênico da água de retorno das lavouras de arroz de Capivarido Sul (Rio Capivari, RS) através do sistema teste Allium cepa*. Monografia, Universidade Federal do Rio Grande do Sul, 41pp.

Pitalua E, Jimenez M, Vermon-Carter EJ, Beristain CI 2010. Antioxidative activity of microcapsules with beetroot juice using gum arabic as wall material. *Food and Bioproc Process* 88:253-258.

Porto BC, Cristianini M 2014. Evaluation of cashew tree gum (*Anacardium occidentale* L.) emulsifying properties. *Lwt-Food Sci and Tech* 59:1325-1331.

Prapajati VD, Jani GK, Naresh GM, Moradiya NG, Randeira NP, Nagar BJ, Naikwadi NN, Variya BC 2013. Galactomannan: A versatile biodegradable seed polysaccharide. *Int J Biol Macromol* 60:83-92.

Radi PA, Terrones MGH 2007. Metabólitos secundários de plantas medicinais. *Rev Bras Farm* 20(2):18-22.

Ramalho IMM 2014. *Biopolímero da Goma de Cajueiro (Anacardium occidentale) como potencial excipiente farmacêutico,* Monografia*,* Universidade Estadual da Paraíba, 27pp.

Ramesh H, Tharanathan RN 1999. Water-extrated polysaccharides of selected cereals and influence of temperature on the extractability of polisaccharides in sorghum. *Food Chem* 64:345-350.

Rao K, Sudarshan RP, Rajasekhar MD, Nagaraju N, Appa Rao CH 2003. Antidiabetic activity of *Terminalia pallida* fruit in alloxan induced diabetic rats. *J Ethnopharmacol* 85(1):169-72.

Ribeiro AJ, De Souza FRL, Bezerra JMNA 2016. Gums' based delivery systems: review on cashew gum and its derivatives. *Carbohydr Polym* 147:188-200.

Ribeiro RV, Bieski IGC, Balogun SO, Martins DTO 2017. Ethnobotanical study of medicinal plants used by Ribeirinhos in the North Araguaia microregion, Mato Grosso, Brazil. *J Ethnopharmacol* 205:69-102.

Rodrigues JF, De Paula RCM, Costa SM 1993. Métodos de Isolamento de Gomas Naturais: Comparação Através da Goma do Cajueiro (*Anacardium occidentale* L). *Polímeros* 3(1):31-36.

Saleem A, Husheem M, Härkönen P, Pihlaja K 2002. Inhibition of cancer cell growth by crude extract and the phenolics of *Terminalia chebula* retz. fruit. *J Ethnopharmacol* 81(3):327-36.

Sekkal N, Taleb-Mokhtari IN, Sekkal-Rahal M, Bleckmann P, Vergoten G 2003. Harmonic dynamics of a- and b-methyl-D-galactopyranoside in the crystalline state. *Spectrochim Acta A Mol Biomol Spectrosc* 59:2883-2896.

Silva TM, Souza ERB, De Paula JAM, Borges LL, Caramori SS 2017. Chemical Characterization and Bioprospecting of Cashew Tree Polyssacharide of Brazilian Cerrado (*Anacardium othonianum* Rizz.), Anarcadiaceae. *Fronteiras: J of Soc Technol and Env Sci* 6(3):230-246.

Silverstein RM, Bassler GC, Morril TC 2013. *Identificação Espectrométrica de Compostos Orgânicos*. 5.ed. Guanabara 2, Rio de Janeiro.

Sudhakar R, Ninge KN, Gowda N, Venu G 2001. Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. *Cytologia* 66:235-239.

Tabarsa M, Anvari M, Joyner HS, Behnam S, Tabarsa A 2017. Rheological behavior and antioxidant activity of a highly acidic gum from Althaea officinalis flower. *Food Hydrocol* 69:432-439.

Varma AJ, Kokane SP, Pathak G, Pradhan SD 1997. Thermal behavior of galactomannan guar gum and its periodate oxidation products. *Carboby Polym* 32:111-114.

Villetti MA, Crespo JS, Soldi MS, Pires ATN, Borsali R, Soldi V 2002. Thermal degradation of natural polymer. *J of Therm Anal Calorim* 67:295-303.

Wolfrom ML, Tompson A 1963. Reduction with sodium borohydride. *Methods* in *carbohydrate chemistry* 2:65-68.

Yamashita FY, Fernandes KF 2012. Biodegradable and bioactive CGP/PVA film for fungal growth inhibition. *Carbohydr Polym* 89:964-970.

Zohuriaan MJ, Shokrolahi F 2004. Thermal studies on natural and modified gums. *Polym Test* 23(5):575-579.

Zuin VG, Budarin VL, De Bruyn M, Shuttleworth PS, Hunt AJ, Pluciennik C, Borisova A, Dodson JR, Parker HL, Clark JH 2017. Polysaccharide-derived mesoporous materials (Starbon®) for sustainable separation of complex mixtures. *Faraday Discuss* 202.

Goma de *Terminalia argentea* Mart. Et. Zucc. (Combretaceae) como Recurso em Processos de Biotecnologia: Química e Toxicidade

RESUMO

Polímeros biodegradáveis estão em áreas como a farmacêutica, alimentos e processos de bioremediação. O isolamento, a caracterização (MEV, FTIR, TGA, Composição de monossacarídeos, Potencial Zeta), atividade antimicrobiana e toxicidade frente a *Artemia salina* e *Allium cepa* L. do exsudato gomoso *in natura* de *Terminalia argentea* (GTa) são aqui apresentados. Os dados mostram morfologia irregular e depressões, bandas em 1770, 2112, 1354 cm^{-1}, indicando grupamentos de polissacarídeos, caráter anfiflitico, termodegradação acima de 300°C e baixa atividade de água (0.6). Os ensaios foram negativos para *S. aureus* e *E. coli*, atóxicos frente aos bioindicadores testados. O GTa apresentou-se de baixo custo, extraído e isolado de forma sustentável, com possibilidade de exploração comercial por comunidades, uma vez que estes biopolímeros apresentam possibilidades de aplicações

como agentes no suporte de imobilização de enzimas, como sensores em diagnósticos, material encapsulante em processos de microencapsulação de fármacos e aditivos em indústrias de alimentos.

Palavras-Chave: Capitão-do-Campo; Biopolímero; Toxicidade Ecológica.

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