Revista Brasileira de Farmacognosia 27 (2017) 220–227

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

Anti-inflammatory, and antinociceptive effects of *Campomanesia adamantium* microencapsulated pulp

Danieli Z. Viscardi[a,*], Vinícius S. de Oliveira[b], Jucicléia da S. Arrigo[a], Ana C. Piccinelli[c], Claudia A.L. Cardoso[d], Iriani R. Maldonade[e], Cândida A.L. Kassuya[c], Eliana J. Sanjinez-Argandoña[f]

[a] *Programa de Pós-graduação em Biotecnologia e Biodiversidade, Faculdade de Ciências Exatas e Tecnologia, Universidade Federal da Grande Dourados, Dourados, MS, Brazil*
[b] *Faculdade de Ciências Exatas e Tecnologia, Universidade Federal da Grande Dourados, Dourados, MS, Brazil*
[c] *Faculdade de Ciências da Saúde, Universidade Federal da Grande Dourados, Dourados, MS, Brazil*
[d] *Curso de Química, Universidade Estadual de Mato Grosso do Sul, Dourados, MS, Brazil*
[e] *Embrapa Hortaliças, Brasília, DF, Brazil*
[f] *Faculdade de Engenharia, Universidade Federal da Grande Dourados, Dourados, MS, Brazil*

ARTICLE INFO

Article history:
Received 1 March 2016
Accepted 7 September 2016
Available online 11 November 2016

Keywords:
Campomanesia adamantium
Inflammation
Hyperalgesia
Microencapsulation
Guavira

ABSTRACT

Guavira fruits have antimicrobial, antioxidant, antinociceptive, and anti-inflammatory activities. Spray drying has been widely used in the food industry presenting good retention in bioactive compounds used to transform the pulp/fruit juice into powder form. Therefore, the present study has evaluated the anti-inflammatory and antinociceptive activities of the microencapsulated pulp of *Campomanesia adamantium* (Cambess.) O.Berg, Myrtaceae, by spray drying. Different groups of mice were treated with the doses of 100 and 300 mg/kg of microencapsulated "guavira" pulp and inflammatory parameters were assessed in a carrageenan paw edema-model and leukocyte migration with pleurisy model, while the antinociceptive activity was assessed using the formalin method and CFA-induced hyperalgesia model. A significant reduction in leukocyte migration and in paw edema was observed in rodents in all time after carrageenan injection for both doses of microencapsulated pulp of *C. adamantium* when compared with control group. Microencapsulated pulp of *C. adamantium* also reduced licking time at the first (nociceptive) and second (inflammatory) phases in the formalin model. In CFA-induced cold and mechanical hyperalgesia, depressive behavior, and knee edema, all parameters analyzed were significantly inhibited by microencapsulated pulp of *C. adamantium*. Microencapsulation by spray drying proved to be a technique that promotes bioavailability and the preservation of bioactive components in guavira pulp.

Introduction

Among the Brazilian Cerrado, there is a native plant species known as *Campomanesia adamantium* (Cambess.) O.Berg, Myrtaceae, (guavira) (Lorenzi, 2000) that is widely found in isolated fields in Midwestern and southeastern Brazil. The fruits grow on small bushes and have specific characteristics, such as bright colors from green to yellow, with a strong and citric aroma (Fernandes et al., 2014). Its fruit has the potential to be used *in natura* in the food industry and as flavoring in the drink industry due to its juiciness, mineral content, fiber, and interesting bioactive substances from the nutritional and functional points of view as phenolic compounds. In addition to their pleasant taste, the fruits are considered a source of vitamin C (Breda et al., 2012), which is an important micronutrient involved in several biological functions in the human body (Pascoal et al., 2014).

In folk medicine "guavira" fruits are used as antirheumatic, antidiarrheal, hypocholesterolemic, anti-inflammatory (Ramos et al., 2007), and to the treatment of cystitis and urethritis (Pascoal et al., 2014). Previous studies with the fruits have observed antimicrobial (Pavan et al., 2009; Cardoso et al., 2010), antioxidant (Coutinho et al., 2010), antinociceptive, and anti-inflammatory activities (Ferreira et al., 2013), as well as apoptotic and antiproliferative activities in PC-3 human prostate carcinoma cells (Fernandes et al., 2014). Phytochemical investigations have found the presence of flavanones and chalcones in the ethyl acetate extract from its fruits (Pavan et al., 2009) and phenolic contents in the ethyl acetate, ethanol, and hexane extracts from the leaves, particularly flavonoids (Coutinho et al., 2010). Ferreira et al. (2013) has demonstrated the presence of myricitrin, quercetin, and myricetin in ethyl acetate in the extract from the leaves of *C. adamantium*. The hydro-alcoholic extract of *C. adamantium* fruit peels has

* Corresponding author.
E-mail: danieliviscardi@ufgd.edu.br (D.Z. Viscardi).

http://dx.doi.org/10.1016/j.bjp.2016.09.007

anti-inflammatory, antihyperalgesic, and antidepressant activities in rodents (Souza et al., 2014).

The fruits of *C. adamantium* are restricted to the period of harvesting (Coutinho et al., 2008). Conservation alternatives to improve the availability of pulp, such as spray drying, has been widely used in the food industry (Tonon et al., 2009, 2013) presenting good retention in bioactive compounds used to transform the pulp/fruit juice into powder form (Chen et al., 2014), allowing prolonged storage and greater stability of the product, giving it a longer shelf life (Souza et al., 2014; Mahdavi et al., 2014). Together with this idea, recent technological advances, such as the microencapsulation technique, have helped to solve these issues and renewed interest in natural products in drug discovery (Lescano et al., 2014).

Phenolic compounds, particularly flavonoids, have free-radical scavenging properties and also inhibit lipid peroxidation. The presence of flavonoids and chalcones in the human diet can reduce the risk of cancers and tumors and also exhibit several activities, such as antibacterial, antifungal, anti-inflammatory, antileishmanial, antimalarial, and anti-HIV protease (Hodek et al., 2012; Tewtrakul et al., 2003; Djeridane et al., 2006; Cabrera et al., 2007; Nowakowska, 2007).

Therefore, the study of microcapsuled fruits of *C. adamantium* enables scientific knowledge of the pharmacological properties of the plant, considering that there may be loss of bioactive compounds and the microencapsulation technique promotes its preservation. Thus, this study aims to evaluate the anti-inflammatory parameters and antinociceptive effects of the microencapsulated pulp of *C. adamantium* (MPCA) in rodents.

Materials and methods

Plant material

Fruits of *Campomanesia adamantium* (Cambess.) O.Berg, Myrtaceae, were collected at "Cerrado," Brazil, on November 2014. A voucher specimen was deposited in the herbarium of the Faculty of Biological Sciences of UFGD (DDMS 4602). Fruits were sanitized and the pulp, peel, and seeds were separated. The pulp was packed in rigid polypropylene containers and stored at −18 °C until use.

High-performance liquid chromatography (HPLC)

The solvents employed were methanol (HPLC grade, Tedia Company, Fairfield, OH, USA) and acetonitrile (HPLC grade, Tedia Company, Fairfield, OH, USA). Samples of the pulp of *C. adamantium* were prepared with 1 g of pulp extract dissolved in 5 ml of methanol in an ultrasound for 20 min. Samples of MPCA were prepared with 5 g of microcapsules that were extracted with 25 ml of methanol in an ultrasound for 20 min and the dried material into a chapel was reconstructed in 5 ml of methanol.

The samples and standards were analyzed using an analytical HPLC (Varian 210) system, with a ternary solvent delivery system and an autosampler. A photodiode array detector was monitored at λ = 200–800 nm. The HPLC column was C-18 (25 cm × 4.6 mm; particle size, 5 μm; Luna, Phenomenex, Torrance, CA, USA), with a small pre-column (2.5 cm × 3 mm) containing the same packing to protect the analytical column. The flow rate and injected volume were 1.0 ml min^{-1} and 20 μl, respectively. All chromatographic analyses were performed at 22 °C.

The elution was conducted using in 0 min, acetonitrile 12%, water 50%, and methanol 38%; in 40 min, acetonitrile 10%, water 10%, and methanol 80%; and in 45 min, returning to the initial condition.

The substances used in HPLC analysis were isolated from the leaves of *C. adamantium*. Compounds were purified by HPLC, resulting in purity between 86% and 96%. The substances were dissolved separately in methanol to a concentration of 10 μg/ml degree chromatographic preparation of stock solutions used in the analysis by HPLC. The standards were easily identified by their UV absorption spectra and retention times. The substances found in the extracts were unambiguously identified by performing coinjection experiments in which aliquots of samples and standards were mixed and diluted to a known volume and analyzed by HPLC.

Microcapsulation of Campomanesia adamantium *fruits*

After preliminary tests and through other studies described in the literature, microcapsules were produced using maltodextrin 8% (DE 20 Maltogill, Cargill, Uberlândia, Brazil), gum Arabic 8% (Synth, Brazil), and chitosan 8% (Purifarma, São Paulo, Brazil) purchased from JKLAB (Química Diagnóstica e Segurança Ltda) (Oliveira et al., 2014). Samples were prepared using 24% encapsulating agent, 16% distilled water, and 60% pulp. The mixture of each formulation was homogenized in an Ultra-Turrax at a speed of 18,000 rpm until complete dissolution of the carrier agent, obtaining samples comprising 30% solids (encapsulating agent and pulp). The atomization process was conducted in a concurrent flow pattern using a mini spray dryer – LM (model MSD 1.0 LABMAQ). Samples were fed into the atomizer at a flow rate of 0.5 l h^{-1} with a 1.2 mm nozzle diameter, air flow of 35 l min^{-1}, and a drying air temperature of 180 °C. The determination of moisture content and ascorbic acid (AOAC, 2000) were performed on fresh and microencapsulated pulp of guavira.

Experimental animals

Male and female Swiss mice (20–25 g) were obtained from Universidade Federal da Grande Dourados (UFGD) biotherium. The animals were kept in collective cages under controlled temperature (23 ± 1 °C) and light conditions (12-h light/dark cycle), and had access to food and water *ad libitum*. The 23/2014 protocol was approved by the Ethics Committee on Animal Use (CEUA/UFGD).

Pleurisy

Different groups of female Swiss mice (n = 5 animals/group) were orally treated with MPCA at doses of 100 and 300 mg/kg or vehicle (0.9% saline solution, also called the control group). The positive control group received dexamethasone subcutaneously at a dose of 1 mg/kg. Pleurisy was induced in experimental groups by intrapleural injection of 100 μl of 1% carrageenan diluted in saline, after 1 h of treatment, as previously described (Velo et al., 1973). The naive group received 100 μl of sterile saline by intrapleural injection. After 4 h, animals were euthanized and the pleural cavity was washed with 1 ml phosphate-buffered saline. An aliquot of 20 μl of lavage (exudate) was collected from the pleural cavity, and diluted with Turck solution (1:20) and used for total leukocyte count in a Neubauer chamber (Kassuya et al., 2009).

Formalin-induced nociception

Sixty min before formalin injection, male Swiss mice (n = 6 animals/group) were divided into groups: dexamethasone (1 mg/kg, subcutaneous route), MPCA (100 and 300 mg/kg, oral route), and vehicle (saline solution, 0.9%, oral route). After respective treatment, 20 μl of saline containing 2.5% of formalin was injected in the right hind paw. Nociceptive response (paw licking) in seconds was evaluated from 0 to 5 min (phase 1 – neurogenic pain) and from 15 to 30 min (phase 2 – inflammatory response) after injection of

formalin in the paw (Kassuya et al., 2009). After that, animals were submitted to cold sensitivity, paw edema measurement.

Open field test

Fifty minutes after oral treatment with MPCA (100 and 300 mg/kg) or vehicle (saline solution, 0.9%, oral route), the mice were placed individually in the center of the arena, and the behavior was quantified for 5 min. The number of "squares" invaded (ambulation) in the center and the periphery of the arena were analyzed. Ambulation was used to evaluate the horizontal movement (Piccinelli et al., 2015).

Carrageenan-induced paw edema

Different groups of male Swiss mice ($n = 5$ animals/group) were orally treated with MPCA (100 and 300 mg/kg), or vehicle (control group). Another group was treated subcutaneously with dexamethasone (1 mg/kg). After 1 h, animals received a solution of 50 μl carrageenan injection (300 μg/paw) in the left hind paw. The other paw received the same volume of sterile saline 0.9%. The paw volume was measured at 1, 2, 3, and 4 h after carrageenan injection with a plethysmometer. The results were expressed as the difference between the left and right paws at each time (Kassuya et al., 2009).

CFA-induced paw inflammation, cold and mechanical hyperalgesia, and depression

Animals were subjected to induction of peripheral inflammation by administration of 20 μl of CFA (Freund's Complete Adjuvant) into the right paw. For 10 days after CFA, different groups of male Swiss mice ($n = 6$/group) were orally treated with MPCA (100 mg/kg). Basal values of mechanical sensitivity were determined before CFA injection. Paw edema was performed as previously described at 1, 2 and 4 h on the first day and once per day for 10 days. In addition, response to acetone (sensitivity to cold), tail suspension (to analyze depression), and knee edema were also performed.

Cold hyperalgesia was measured by the acetone test as described previously (25). A needle connected to a syringe was used to drop 30 μl of acetone on the paw and the duration (in seconds) of the paw withdrawal was recorded. Minimal and maximal cut-offs were assigned at 0.5 s and 20 s, respectively. Paw withdrawal due to locomotion or weight shifting were not counted and such trials were repeated (Ferreira et al., 2013).

Mechanical sensitivity was measured with Electronic analgesimeter (Aquino et al., 2015) at 0 (basal values), 1, 2, and 4 h after the injection on the first day and once per day for 10 days. Oral treatment was administered daily for 10 days after CFA injection.

Thickness paw edema was assessed using a plethysmometer 30 min before any treatment and after 1 h of formalin injection. The results were expressed in microliters as the difference between the baseline and post-injection edema values, with modifications of Kassuya et al. (2009). On the 10th day after CFA injection, assessment of knee edema was performed in mice, using a micrometer.

Tail suspension test is a depression model that assesses immobility of mice. Mice were individually suspended on an acrylic box with their tails attached with tape. The behavior was filmed for 6 min and the duration of immobility was measured in seconds.

Statistical analyses

The results are expressed as mean ± standard error of the mean. For comparison of results between experimental groups, analysis of variance (one-way and two-way ANOVA) was used, followed by the Newman–Keuls or Bonferroni test. The number of animals per group is indicated in the legends. Statistical differences were considered significant at $p < 0.05$. Asterisk (*) or (#) denotes a significant difference between groups.

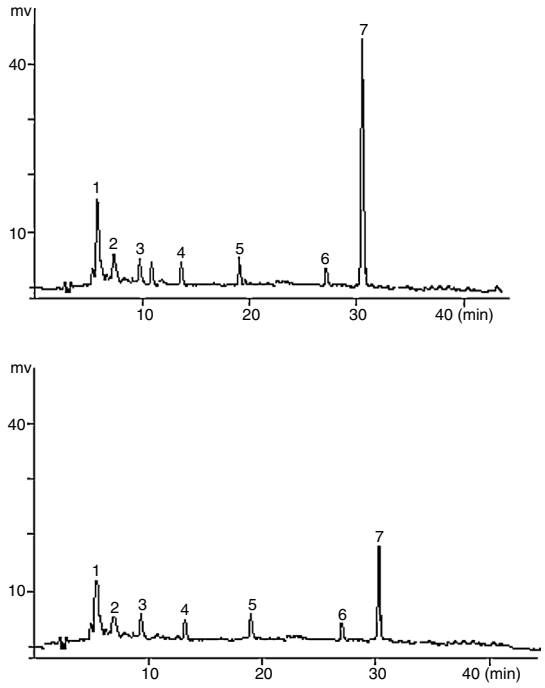

Fig. 1. Chromatogram of the pulp and microcapsules of *Campomanesia adamantium* obtained by HPLC. Identified substances: 3,5,7,3′,4′,5′-hexahydroxy-flavonol (peak 1), 3,5,7,3′,4′,5′-hexahydroxy-flavonol-3-*O*-α-L-arabinofuranoside (Peak 2), 3,5,7,3′,4′,5′-hexahydroxy-flavonol-3-*O*-α-L-raminopyranoside (peak 3), 7-dihydroxy-5-metoxiflavanone (peak 4), 6-methyl-7-hydroxy-5-metoxiflavanone (peak 5), 2′,4′-dihydroxy-6′-metoxichalcone (peak 6), and 2′,4′-dihydroxy-5′-methyl-6′-metoxichalcone (peak 7).

Results

Analyses by high-performance liquid chromatography in the pulp and the *C. adamantium* microcapsules identified the

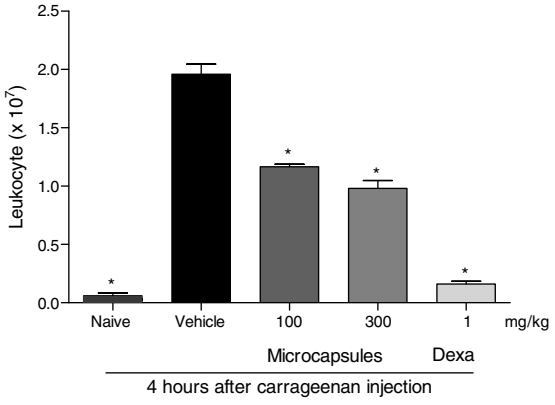

Fig. 2. Effect of oral administration of the pulp and microcapsules of *Campomanesia adamantium* on the inhibition of the leukocyte migration at both doses tested on pleurisy test. Mice were treated 1 h before an intrapleural injection of carrageenan, with microcapsules (100 or 300 mg/kg), dexamethasone (DEXA, 1 mg/kg, *s.c.*), or saline solution (vehicle). Naive group, also treated with saline, received an intrapleural injection of sterile saline. Each bar represents the mean ± SEM of five animals. *$p < 0.001$ when compared to control group. Differences between groups were analyzed by analysis of variance (one-way ANOVA) followed by the Newman–Keuls. test.

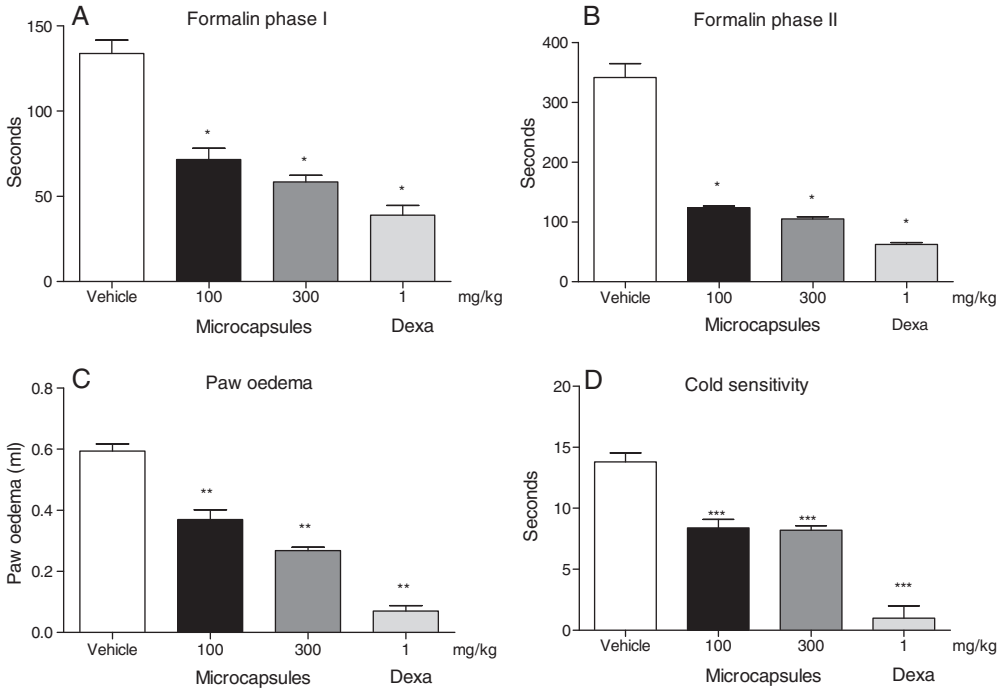

Fig. 3. Effect of the pulp and microcapsules of *Campomanesia adamantium* on formalin-induced paw licking or biting in mice and on formalin-induced cold sensitivity and edema induced by formalin. (A, B) The essential oil of microcapsules at doses of 100 and 300 mg/kg presented antinociceptive effects in phase I and II test. (C) Edema induced by formalin. (D) Effect of the MPCAon cold sensitivity after treatment with microcapsules cold sensitivity with acetone in mice. Each bar represents the mean ± SEM of six animals. $^{*}p < 0.001$ when compared to the control group. Differences between groups were analyzed by analysis of variance (one-way ANOVA) followed by the Newman–Keuls test.

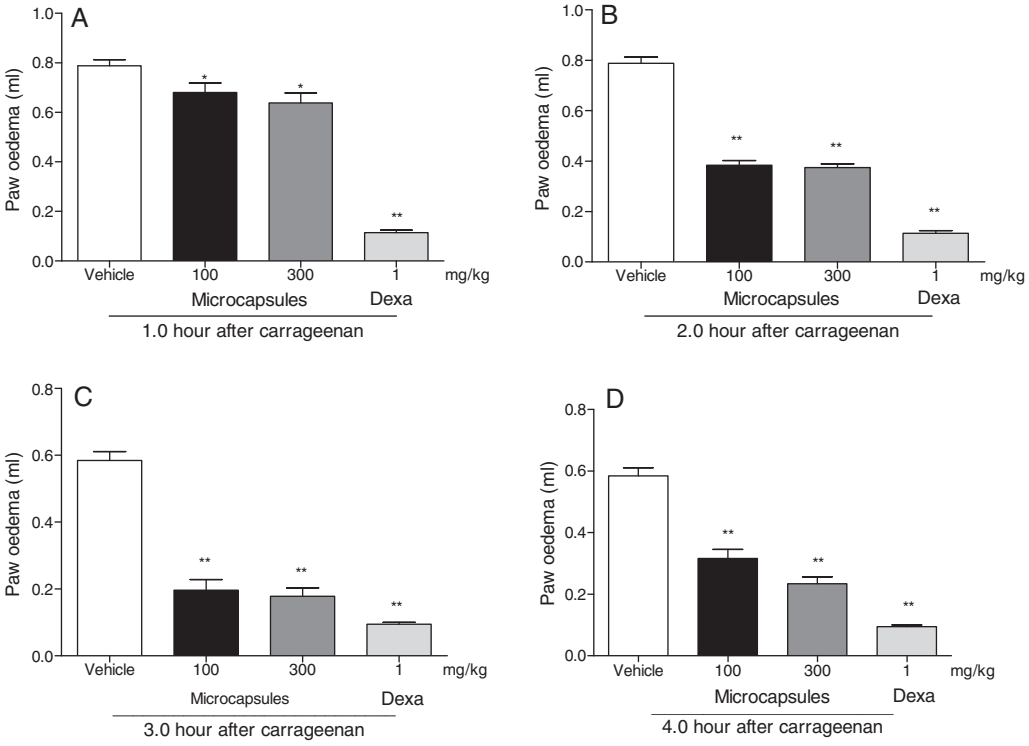

Fig. 4. Effect of the pulp and microcapsules of *Campomanesia adamantium* in carrageenan-induced paw edema in mice. Animals received microcapsules (100 or 300 mg/kg, *p.o.*) or control (vehicle) or dexamethasone (DEXA, 1 mg/kg, *s.c.*) and after 1 h, an intraplantar injection of carrageenan (300 μg/paw). Graphics (A), (B), (C), and (D) represent the evaluation of paw edema after 1, 2, 3, and 4 h, respectively, after carrageenan injection. Each bar represents the mean ± SEM of five animals. $^{*}p < 0.05$, $^{**}p < 0.001$ when compared with the control group. Differences between groups were analyzed by analysis of variance (one-way ANOVA) followed by the Newman–Keuls test.

substances: 3,5,7,3′,4′,5′-hexahydroxy-flavonol (peak 1), 3,5,7,3′,4′,5′-hexahydroxy-flavonol-3-*O*-α-L-arabinofuranoside (peak 2), 3,5,7,3′,4′,5′-hexahydroxy-flavonol-3-*O*-α-L-raminopyranoside (peak 3), 7-dihydroxy-5-metoxiflavanone (peak 4), 6-methyl-7-hydroxy-5-metoxiflavanone (peak 5), 2′,4′-dihydroxy-6′-metoxichalcone (peak 6), and 2′,4′-dihydroxy-5′-methyl-6′-metoxichalcone (peak 7) (Fig. 1).

MPCA prevented migration of leukocytes to the pleural cavity induced by carrageenan

The oral administration of *C. adamantium* microencapsulated pulp significantly inhibited the leukocyte migration at all doses tested (100 and 300 mg/kg), being greater at dose of 100 mg/kg with maximal inhibition of 50 ± 4% compared to controls. The microencapsulated also showed significant activity, reducing the protein extravasation at both doses tested, but with better response at dose of 300 mg/kg with maximum inhibition of 67 ± 3%. For the positive control the inhibition was 91 ± 2% (Fig. 2).

MPCA prevented nociception, cold sensitivity, and neurogenic edema induced by formalin in rats

MPCA (Fig. 3A, Phase I) produced significant antinociceptive effects in the first phase when compared with the control group. At the dose of 100 mg/kg, maximum inhibition was 59 ± 2%, while at the dose of 300 mg/kg, it was 63 ± 3%, and for dexamethasone, it was 73 ± 2%.

MPCA at doses of 100 and 300 mg/kg significantly reduced licking time in the second phase of the formalin test in rats (Fig. 3B, Phase II). At a dose of 100 mg/kg, maximum inhibition was 72 ± 1%, at a dose of 300 mg/kg, it was 70 ± 2%, and for dexamethasone, it was 85 ± 3%.

MPCA caused a reduction in paw edema induced by formalin. Fig. 3C shows the results of paw edema with maximal inhibition of 69 ± 1% for MPCA at the dose of 300 mg/kg, 64 ± 3% at the dose of 100 mg/kg, and 87 ± 2% for dexamethasone.

MPCA (100 and 300 mg/kg) significantly attenuated the duration of cold hypersensitivity after formalin injection. Animals almost did not move and raised their paws a few times after acetone application. At a dose of 100 mg/kg, maximum inhibition was 55 ± 2%, at a dose of 300 mg/kg, it was 60 ± 2%, and for dexamethasone, 86 ± 3%. Furthermore, hypersensitive response to cold was <10 s (Fig. 3D).

Open field results demonstrated that the orally administered microcapsules were not capable of reducing locomotor activity when compared with the control group (results not shown).

MPCA prevented carrageenan-induced paw edema

An hour after the carrageenan-induced inflammation, the control group continued to show edema, whereas the groups treated with microcapsules at doses of 100 and 300 mg/kg showed a significant decrease in edema compared to the control group (Fig. 4A) and this reduction continued after the second, third and fourth hour of observation (Fig. 4B–D). Fig. 4 shows paw edema was also inhibited at all times, and maximal inhibition at the dose of 100 mg/kg was 52 ± 2%, 63 ± 3%, 86 ± 3%, and 77 ± 2%, after 1, 2, 3, and 4 h, respectively. The inhibitions, dose of 300 mg/kg, were 53 ± 2%, 68 ± 3%, 86 ± 2%, and 80 ± 3% after 1, 2, 3, and 4 h, respectively. The animals treated with dexamethasone, the positive control, showed a significant reduction at all time points, with inhibitions of 93 ± 7% after 2 h and 85 ± 5% after 4 h.

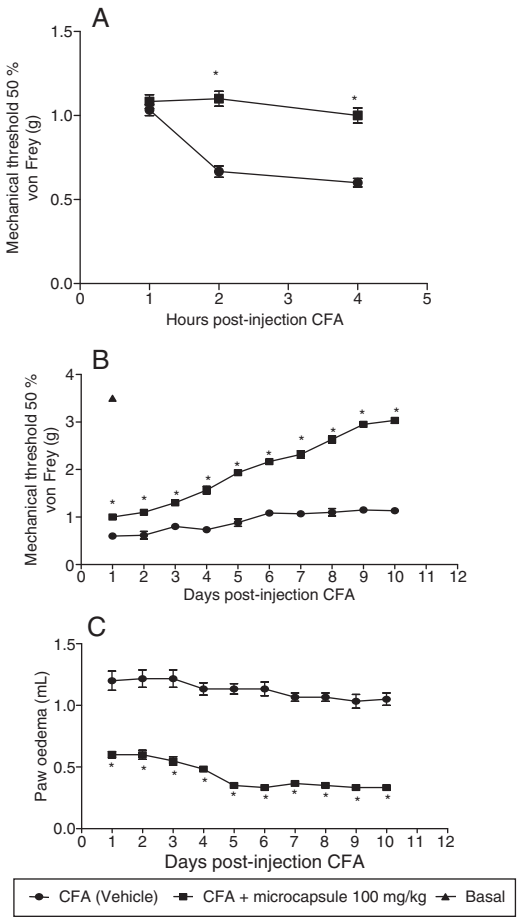

Fig. 5. Effect of the oral administration of microencapsulated pulp of *Campomanesia adamantium* (MPCA) (100 mg/kg) on mechanical hyperalgesia and paw edema in mice. (A) Animals received the MPCA (10 m and 300 mg/kg, *p.o.*) or vehicle, and after 1 h, 20 μl of CFA (Freund's Complete Adjuvant) injection in the right hind paw. Mechanical sensitivity was measured with von Frey analgesimeter at 1, 2, and 4 after the injection on the first day and (B) once a day for 10 days. (C) Effect of oral administration of MPCAon CFA-induced paw edema. Paw edema was performed as previously described once a day during 10 days. Each bar represents the mean ± SEM of six animals. $^{*}p < 0.001$ when compared to the control group. Differences between groups were analyzed by analysis of variance (two-way ANOVA) followed by the Bonferroni tests.

MPCA fruits prevented CFA-induced mechanical and cold hyperalgesia and knee edema

The results of mechanical hyperalgesia assessed by analgesimeter are shown in Fig. 5. Treatment with CFA and MPCA demonstrated a significant increase in paw withdrawal threshold after 10 days of treatment when compared with the control group. Microcapsules of *C. adamantium* pulp presented an effect against mechanical hyperalgesia at all days analyzed (Fig. 5A). On the first day, significant results were observed after 2 and 3 h of CFA injection at a dose of 100 mg/kg with maximal inhibition of 61 ± 1% after 2 h and 55 ± 2% after 3 h. On day 9, they demonstrated maximal inhibition of 86 ± 2% and reduction of edema on day 6 with maximal inhibition of 87 ± 3% (Fig. 5B). Significant differences in mechanical thresholds were observed in the group treated with CFA and microencapsulated pulp of *C. adamantium*. These results are very similar to those of the control group.

Administration of MPCA (100 mg/kg) significantly attenuated the duration of cold hypersensitivity. The treated group almost did not move and raised their paws a few times with acetone application. Furthermore, the hypersensitive response to cold in the treated group was <10 s, there was a significant decrease in

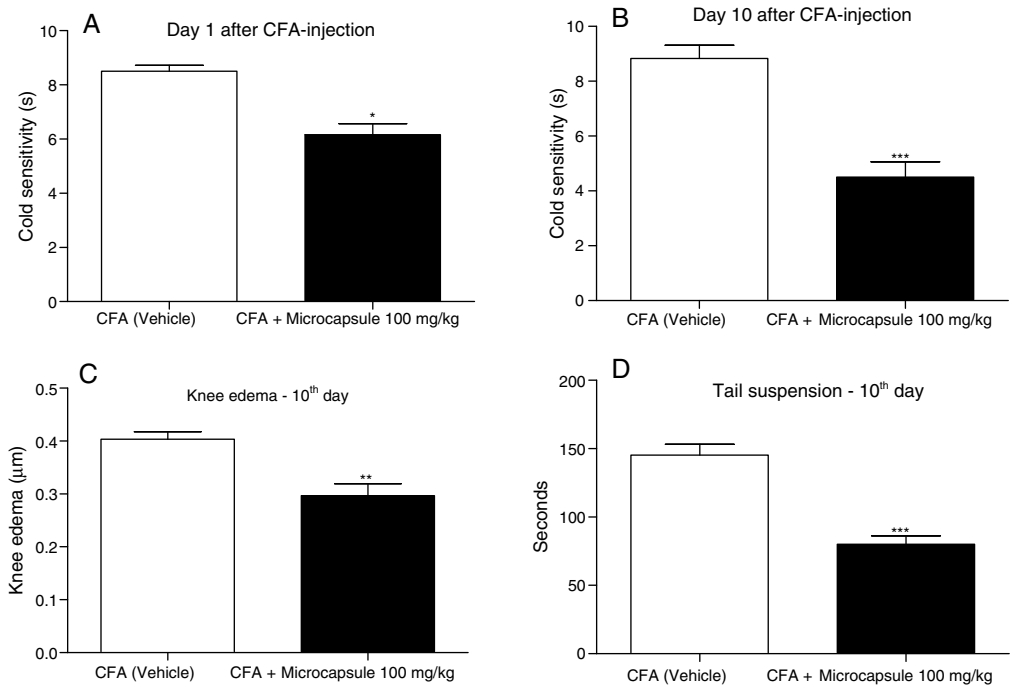

Fig. 6. (A) Effect of microencapsulated pulp of *Campomanesia adamantium* (MPCA) on cold sensitivity after treatment with microcapsules at the dose of 100 mg/kg and an injection of CFA (Freund's Complete Adjuvant) in the right hind paw. (B) Effect of MPCA on cold sensitivity after treatment with microcapsules on the 10th days after CFA injection. (C) Effect of MPCA on knee edema at the 10th day after CFA injection. The test was performed in mice using a micrometer. (D) Effect of MPCA on tail suspension test. Mice were individually suspended on an acrylic box with their tails attached with tape. Behavior has been filmed for 6 min and the duration of immobility was measured in seconds. Each bar represents the mean ± SEM of six animals. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ when compared to the control group. Differences between groups were analyzed by analysis of variance (one-way ANOVA) followed by the Newman–Keuls test.

movements when compared with the control (Fig. 6A), and one day after CFA injection, the maximum inhibition was 44 ± 2%. There was a decrease in licking time after 10 days. On the first day, licking time was 6 s and on the last day, it was 4 s, while on the 10th day after applying the CFA the maximum inhibition was 62 ± 3% (Fig. 6B).

For knee edema test after 10 days we could see maximum inhibition of 63 ± 1% (Fig. 6C) and for tail suspension test maximum inhibition was 67 ± 2% (Fig. 6D), when compared with the control group.

Discussion

Despite its economic and cultural importance in the Brazilian Cerrado, there are few clinical trials with the species *C. adamantium*. Many ethnopharmacological studies have reported pharmacological efficiency (Souza et al., 2014). Myrtaceae family plants are distributed throughout the Cerrado region of Brazil and some species have been used to treat pain, inflammation and other diseases. These anti-inflammatory properties are attributed to flavonoids and chalcones, present as the main constituents of the extract of this plant (Pavan et al., 2009; Ramos et al., 2007; Coutinho et al., 2009) and fruit pulp. Flavonoids (Coutinho et al., 2008) are widely distributed among plants and exhibit pharmacological effects on the inflammatory process.

The present study demonstrated the anti-inflammatory and antinociceptive analyses of *C. adamantium* microencapsulated pulp. Experimental data demonstrated *C. adamantium* microencapsulated pulp inhibited leukocyte migration, inflammatory, neurogenic pain and edema, CFA-induced paw inflammation and mechanic hypersensitivity measurement suggesting their use as a nutraceutical or pharmacological agent.

Previous results from our group have demonstrated that the hydroalcoholic extract of the fruit peel of *C. adamantium* exhibited anti-inflammatory and antihypernociceptive effects in pleurisy and mechanical nociception (Souza et al., 2014). In the present study, the results have indicated the anti-inflammatory and antinociceptive actions of the microencapsulated pulp *C. adamantium*. Experimental data have demonstrated that MPCA inhibited leukocyte migration, inflammatory and neurogenic pain, and edema, suggesting their use as a nutraceutical or pharmacological agent. Those findings corroborate with the popular use of *C. adamantium* fruits as anti-inflammatory (Ramos et al., 2007). The anti-inflammatory activity of MPCA in acute inflammation was evaluated by induction of a carragenan-induced pleurisy model. This is a classic test to evaluate this type of inflammation and the formation of a pleural exudate in the cavity is characterized by infiltration of polymorphonuclear leukocytes and the release of several important chemical mediators in the inflammatory process (Oliveira et al., 2014). Anti-inflammatory drugs, such as indomethacin and dexamethasone, inhibit leukocyte migration between 3 and 6 h after carrageenan administration.

Treatment with MPCA 1 h before carrageenan injection was able to significantly decrease the total leukocyte recruitment in the pleural cavity. This result was also confirmed by Ferreira et al. (2013) and Souza et al. (2014).

In the paw edema test, there was a significant decrease in swelling after 3 h from the time of administration when compared with the control group, as previously observed (Ferreira et al., 2013). According to Souza et al., this probably happens because of a reduction in local vascular permeability. Thus, we concluded that *C. adamantium* promotes a reduction in cell leakage, leading to the consequent reduction of proinflammatory mediators.

HPLC analyses of *C. adamantium* microcapsules have identified 2′,4′-dihydroxy-5′-methyl-6′-metoxichalcone as a major constituent, which corroborates the data of Pascoal et al., the phytochemical study of ethyl acetate fraction, which led to

the isolation and identification of the chalcone (2′,4′-dihydroxy-6′-metoxichalcona), which showed promising antiproliferative activity against some of the human tumor cell lines studied.

Some of these compounds and substances of the class of chalcones have been described as natural analgesic agents in the literature (Raygude et al., 2012) and presented chemopreventive and antitumor effects (Rahman, 2012). Ferreira et al. has reported myricetin as a potential compound responsible for antinociceptive action of *C. adamantium* extract. Maybe myricetin and other compounds found in this species exhibit antihyperalgesic and anti-inflammatory effects.

Phytochemical evaluation has identified the presence of three types of flavonols, two of flavonones, and two of chalcones, which presented the highest chromatographic profile of both pulp microcapsules. According to Dewick (2005), the increase in chalcone content may be related to a biosynthetic strategy for the formation of flavonones that could interfere in the production of secondary metabolites of plants as a defense strategy of these plants (Harbone, 1994). Furthermore, according to Coutinho et al. (2010) this increase may be due to the accumulation of flavonoids on the surface of the leaf as a form of protection from sunlight or as a plant defense mechanism against insects.

It is known that flavonoids are plant polyphenol compounds that present analgesic and anti-inflammatory properties by inhibiting enzymes involved in inflammation and pain in several parts of the nervous system (Coutinho et al., 2010). In the inflamed tissue, flavonoids are able to inhibit cyclooxygenase, prevent prostaglandin formation and TNF secretion, which are responsible in stimulating pain receptors in the brain. Flavonoids also decrease analgesic activity by inhibiting nitric oxide synthesis and NO production.

We know that the chemistry of natural products appears to be a promising alternative and has attracted the increasing attention of scientists to search for new pharmacologically active agents, mainly to improve the treatment of pain. Therefore, the present investigation of the administration of MPCA (100 mg/kg per day for 10 days) is a new approach and has a beneficial effect on the treatment of neuropathy because of its multiple effects: anti-inflammatory, as indicated by the CFA-induced paw edema test, and antinociceptive, as indicated by the CFA-induced cold sensitivity, induced knee edema, and tail suspension tests.

In this study, we have observed that the microencapsulation method of spray drying has great applicability, being characterized as an effective method and one of extreme importance in the preservation of several nutritional components, protecting against the most aggressive methods of processing. The maintenance of bioactive compounds, such as flavonoids in microencapsulated pulp from *C. adamantium*, can also be observed in this work.

MPCA has demonstrated anti-inflammatory and antihyperalgesic activities, probably due to the presence of bioactive compounds that interfere with inflammatory parameters, supporting the use of this plant part in folk medicine. In addition, the microcapsules retained the stability of the bioactive compounds, enhancing the development of new products based on natural products. Additional studies are needed to elucidate the exact mechanism of action and the compounds responsible for this activity.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

DZV (PhD student): contributed to the collection and identification of plant samples, performing the laboratory work, data analysis, and drafting of the article. VSO: contributed to the development of *C. adamantium* microcapsules. JSA: contributed to carrying out the *in vivo* laboratory work. ACP: contributed to data analysis and drafted the article. CALC: Professor responsible for high-performance liquid chromatography. CALK: design and supervision of the *in vivo* experiments. IRM: contributed to critical reading of the manuscript. EJSA: designed the study, supervised the laboratory work, and contributed to critical reading of the manuscript. All of the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors also wish to thank the Fundação de Amparo à Pesquisa do Estado do Mato Grosso do Sul (FUNDECT) for the financial support, through the projects FUNDECT/CAPES n° 45/2014 – PAPOS REDE PRÓ-CENTROOESTE – FASE II – N° 23/200.622/2014 and the CNPq.

References

Aquino, D.F.S., Piccinelli, A.C., Soares, F.L., Arena, A.C., Salvador, M.J., Kassuya, C.A., 2015. Anti-hyperalgesic and anti-inflammatory activity of alternanthera maritima extract and 2″-*O*-α-L-rhamnopyranosylvitexin in mice. Inflammation 1, 1–10.

AOAC, 2000. Official Methods of Analysis of AOAC International. Association of Official Analytical Chemists. AOAC, Maryland.

Breda, C.A., Sanjinez-Argandoña, E.J., Correia, C.A.C., 2012. Shelf life of powdered *Campomanesia adamantium* pulp in controlled environments. Food Chem. 135, 2960–2964.

Cabrera, M., Simoens, M., Falchi, G., Lavaggi, M.L., Piro, O.E., Castellano, E.E., 2007. Synthetic chalcones, flavanones, and flavones as antitumoral agents: biological evaluation and structure–activity relationships. Bioorg. Med. Chem. 15, 3356–3367.

Cardoso, C.A., Salmazzo, G.R., Honda, N.K., Prates, C.B., Vieira, M.C., Coelho, R.G., 2010. Antimicrobial activity of the extracts and fractions of hexanic fruits of *Campomanesia* species (Myrtaceae). J. Med. Food 13, 1273–1276.

Chen, Q., Zhou, Y., Liu, X., Wu, X., Chen, R., 2014. Multi-objective optimization of spray drying of jujube (*Zizyphus jujuba* Miller) powder using response surface methodology. Food Bioprocess Technol. 7, 1807–1818.

Coutinho, I.D., Cardoso, C.A.L., Ré-Poppi, N., Melo, A.M., Vieira, M.C., Honda, N.K., Coelho, R.G., 2009. Gas Chromatography–Mass Spectrometry (GC–MS) evaluation of antioxidant and antimicrobial activities of essential oil of *Campomanesia adamantium* (Cambess.) O Berr. (Guavira). Braz. J. Pharm. Sci. 45, 767–776.

Coutinho, I.D., Coelho, R.G., Kataoka, V.M.G., Honda, N.K., Silva, J.R.M., Vilegas, W., 2008. Determination of phenolic compounds and evaluation of antioxidant capacity of *Campomanesia adamantium* leaves. Eclet. Quim. 33, 53–60.

Coutinho, I.D., Kataoka, V.M.G., Honda, N.K., Coelho, R.G., Vieira, M.C., Cardoso, C.A.L., 2010. Influência da variação sazonal nos teores de flavonóides e atividade antioxidante das folhas de *Campomanesia adamantium* (Cambess.) O. Berg, Myrtaceae. Rev. Bras. Farmacogn. 20, 322–327.

Dewick, P.M., 2005. Medicinal Natural Products: A Biosynthetic Approach. John Wiley & Sons, England.

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., Vidal, N., 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem. 97, 654–660.

Fernandes, T.O., Ávila, R.I., Moura, S.S., Ribeiro, G.M., Naves, M.M.V., Valadares, M.C., 2014. *Campomanesia adamantium* (Myrtaceae) fruits protect HEPG2 cells against carbon tetrachloride-induced toxicity. Toxicol. Rep. 2, 184–193.

Ferreira, L.C., Grabe-Guimarães, A., de Paula, C.A., Michel, M.C.P., Guimarães, R.G., Rezende, S.A., Filho, J.D.S., Saúde-Guimarães, D.A., 2013. Anti-inflammatory and antinociceptive activities of *Campomanesia adamantium*. J. Ethnopharmacol. 145, 100–108.

Harbone, J.B., 1994. The Flavonoids-advances in Research Since 1986. Chapman & Hall, London.

Hodek, P., Trefil, P., Stiborová, M., 2012. Flavonoids–potent and versatile biologically active compounds interacting with cytochromes P450. Chem. Biol. Interact. 139, 1–21.

Kassuya, C.A., Cremoneze, A., Barros, L.F., Simas, A.S., Lapa, F. da R., Mello-Silva, R., Stefanello, M.E., Zampronio, A.R., 2009. Antipyretic and anti-inflammatory properties of the ethanolic extract, dichloromethane fraction and costunolide from *Magnolia ovate* (Magnoliaceae). J. Ethnopharmacol. 124, 369–376.

Lescano, C.H., Iwamoto, R.D., Sanjinez-Argandon, E.J., 2014. Diuretic and anti-inflammatory activities of the microencapsulated acrocomia aculeata (Arecaceae) oil on Wistar rats. J. Med. Food 18, 656–662.

Lorenzi, H., 2000. Árvores Brasileiras: Manual de Identificacão e Cultivo de Plantas Arbóreas Nativas do Brasil. Instituto Plantarum, Nova Odessa.

Mahdavi, S.A., Jafari, S.M., Ghorbani, M., Assadpoor, E., 2014. Spray-drying microencapsulation of anthocyanins by natural biopolymers: a review. Dry Technol. 32, 509–518.

Nowakowska, Z., 2007. A review of anti-infective and anti-inflammatory chalcones. Eur. J. Med. Chem. 42, 125–137.

Oliveira, V.S., Sanjinez-Argandona, E.J., Alves, A.V., 2014. Retention of vitamin C in microencapsulated Guavira pulp by spray drying. Revista Facultad Nacional de Agro 67, 202–204.

Pascoal, A.C.R.F., Ehrenfried, C.A., Lopez, B.G.C., Arauj, T.M., Pascoal, V., Gilioli, R., Pavan, F.R., Leite, C.Q.F., Coelho, R.G., Coutinho, I.D., Honda, N.K., Cardoso, C.A.L., 2014. Antiproliferative activity and induction of apoptosis in PC-3 cells by the chalcone cardamonin from *Campomanesia adamantium* (Myrtaceae) in a bioactivity-guided study. Molecules 19, 1843–1855.

Pavan, F.R., Leite, C.Q.F., Coelho, R.G., Coutinho, I.D., Honda, N.K., Cardoso, C.A.L., et al., 2009. Evaluation of anti-mycobacterium tuberculosis activity of Campomanesia adamantium(Myrtaceae). Quim Nova 32, 1222–1226.

Piccinelli, A.C., Santos, J.A., Konkiewitz, E.C., Oesterreich, S.A., Formagio, A.S.N., Croda Julio Ziff, E.B., Kassuya, C.A.L., 2015. Antihyperalgesic and antidepressive actions of (R)-(+)-limonene, α-phellandrene, and essential oil from *Schinus terebinthifolius* fruits in a neuropathic pain model. Nutr. Neurosci. 18, 217–224.

Rahman, M.A., 2012. Chalcone: a valuable insight into the recent advances and potential pharmacological activities. Chem. Sci. J. 29, 1–16.

Ramos, D.D., Cardoso, C.A.L., Yamamoto, N.T., 2007. Avaliação do potencial citotóxico e atividade antioxidante em *Campomanesia adamantium* (Cambess.) O. Berg (Myrtaceae). Rev. Bras. Biocienc. 5, 774–776.

Raygude, K.S., Kandhare, A.D., Ghosh, P., Ghule, A.E., Bodhankar, S.L., 2012. Evaluation of ameliorative effect of quercetin in experimental model of alcoholic neuropathy in rats. Inflammopharmacology 20, 331–341.

Souza, J.C., Piccinelli, A.C., Aquino, D.F.S., Souza, V.V., Schmitz, W.O., Traesel, G.K., Cardoso, C.A.L., Kassuya, C.A.L., Arena, A.C., 2014. Toxicological analysis and antihyperalgesic, antidepressant, and anti-inflammatory effects of *Campomanesia adamantium* fruit barks. Nutr. Neurosci., http://dx.doi.org/10.1179/1476830514Y.0000000145.

Tewtrakul, S., Subhadhirasakul, S., Puripattanavong, J., Panphadung, T., 2003. HIV-1 protease inhibitory substances from *Boesenbergia pandurate* Holtt. Anim. Sci. Technol. 25, 503–508.

Tonon, R.V., Brabet, C., Hubinger, M.D., 2009. Influência da temperatura do ar de secagem e da concentração de agente carreador sobre as propriedades físico-químicas do suco de açaí em pó. Cien. Tec. Aliment. 9, 444–450.

Tonon, R.V., Brabet, C., Hubinger, M.D., 2013. Aplicação da secagem por atomização para a obtenção de produtos funcionais com alto valor agregado a partir do açaí. Inclusão Social 6, 70–71.

Velo, G.P., Dunn, C.J., Giroud, J.P., Timsit, J., Willoughby, D.A., 1973. Distribution of prostaglandins in inflammatory exudate. J. Pathol. 11, 149–158.