

# Phytochemical and biological investigations of *Caryocar brasiliense* Camb

[Estudios fitoquímicos y biológicos sobre *Caryocar brasiliense* Camb]

Jociani ASCARI, Jacqueline Aparecida TAKAHASHI, Maria Amélia Diamantino BOAVENTURA\*

Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, CEP 31270-970. Belo Horizonte – MG, Brasil.

## Abstract

*Caryocar brasiliense* epicarp and external mesocarp were chemically and biologically evaluated. From the phytochemical study, ethyl gallate, 5-hydroxyfurfural, gallic acid, methyl shikimate, and mixtures of  $\beta$ -D-fructopyranose and  $\beta$ -D-fructofuranose,  $\alpha$ - and  $\beta$ -D-glucose, lupeol and oleic acid and  $\beta$ -sitosterol, stigmasterol and oleic acid were isolated and spectroscopically identified by NMR (1D and 2D). Tests on the antioxidant, allelopathic and antimicrobial activities were carried out for the crude extract, fractions and pure compounds. Extract and pure compounds showed good activities in all bioassays.

**Keywords:** *Caryocar brasiliense* Camb.; phenolic compounds; triterpenes; antimicrobial; antioxidant; allelopathic activity

## Resumo

El epicarpo y el mesocarpio externo de *Caryocar brasiliense* Camb. fueron evaluados química y biológicamente. Del estudio fitoquímico, fueron aislados e identificados por RMN (1D y 2D) galato de etilo, 5-hidroximetilfurfural, ácido gálico, chiquimato de metilo y mezclas de  $\beta$ -D-fructopiranososa y  $\beta$ -D-fructofuranosa,  $\alpha$ - y  $\beta$ -D-glucosa, lupeol y ácido oléico,  $\beta$ -sitosterol, estigmasterol y ácido oléico. Fueron realizados ensayos de evaluación del efecto anti-oxidante, anti-microbiano y alelopático para el extracto etanólico crudo, fracciones y compuestos puros, los cuales presentaron buena actividad.

**Palavras Chave:** *Caryocar brasiliense* Camb.; compuestos fenólicos; triterpenos; actividad anti-oxidante; actividad antimicrobiana; actividad alelopática.

**Recibido | Received:** August, 9, 2009.

**Aceptado en Versión Corregida | Accepted in Corrected Version:** October 26, 2009.

**Publicado en Línea | Published Online:** December 15, 2009.

**Declaración de intereses | Declaration of interests:** authors have no competing interests.

**Financiación | Funding:** CNPq, JAT and MADB for grants. FAPEMIG, for financial support.

**This article must be cited as:** . Jociani Ascari, Jacqueline Aparecida Takahashi, Maria Amélia, Diamantino Boaventura. 2010. Phytochemical and biological investigations of *Caryocar brasiliense* Camb. Bol Latinoam Caribe Plant Med Aromat 9(1):20 – 28. {EPub 15 Dec 2009 }.

\*Contactos | Contacts: [dianadb@netuno.lcc.ufmg.br](mailto:dianadb@netuno.lcc.ufmg.br)



BLACPMA es una publicación de la [Cooperación Latinoamericana y Caribeña de Plantas Medicinales y Aromáticas](#)

This is an open access article distributed under the terms of a Creative Commons Attribution-Non-Commercial-No Derivative Works 3.0 Unported Licence. (<http://creativecommons.org/licenses/by-nc-nd/3.0/>) which permits to copy, distribute and transmit the work, provided the original work is properly cited. You may not use this work for commercial purposes. You may not alter, transform, or build upon this work. Any of these conditions can be waived if you get permission from the copyright holder. Nothing in this license impairs or restricts the author's moral rights.

Este es un artículo de Acceso Libre bajo los términos de una licencia "Atribución Creativa Común-No Comercial-No trabajos derivados 3.0 Internacional" (<http://creativecommons.org/licenses/by-nc-nd/3.0/deed.es>) Usted es libre de copiar, distribuir y comunicar públicamente la obra bajo las condiciones siguientes: Reconocimiento. Debe reconocer los créditos de la obra de la manera especificada por el autor o el licenciador (pero no de una manera que sugiera que tiene su apoyo o apoyan el uso que hace de su obra). No comercial. No puede utilizar esta obra para fines comerciales. Sin obras derivadas. No se puede alterar, transformar o generar una obra derivada a partir de esta obra. Al reutilizar o distribuir la obra, tiene que dejar bien claro los términos de la licencia de esta obra. Alguna de estas condiciones puede no aplicarse si se obtiene el permiso del titular de los derechos de autor. Nada en esta licencia menoscaba o restringe los derechos morales del autor.

## INTRODUCTION

Caryocaraceae is a small botanic family widely distributed in Central and South America. It is constituted by two genera, *Caryocar* and *Anthodicus*, which include 25 species. *Caryocar* genus presents the higher number of species (16), being the most economically important since their very nutritive fruits are used as source of edible oils and in the preparation of juices and liqueurs (Prance, 1990).

*Caryocar brasiliense* Camb., known in Brazil as pequizeiro, deserves a distinguished position due to the commercial, nutritional and gastronomic importance of its fruit, named pequi. Pequi is a spherical green fruit, presenting 1-4 segments. Its structure is composed by an epicarp (very thin peel), an external pulpy mesocarp, internal mesocarp (light-yellow, pulpy, rich in oil, vitamins and proteins), that involve a layer of thin and rigid endocarp spines (approximately 2-5 mm large) and white nut (seed). Together, internal mesocarp, needle endocarp and seed constitute one segment (Damiani, 2006). The fruit is used in Central Brazil culinary; fruits and leaves of *C. brasiliense* are used in the folk medicine to treat cold, edema, bronchitis, cough, burns and is also used as a scaring agent (Vieira and Martins, 2000; Magalhães et al., 1988).

From *C. brasiliense* leaves  $\beta$ -amyrin, oleanoic and ellagic acids and a mixture of  $\beta$ -sitosterol and stigmasterol were isolated. Edible pulp showed to be rich in vitamins A, C, riboflavin, thiamin and carotenoids (Azevedo and Rodriguez, 2004). The oil extracted from *C. brasiliense* fruits presented several biological properties such as anti-fungal (Passos et al., 2002), against *Trypanosoma cruzi* (Herzog-Soares et al., 2002) and *Biomphalaria glabrata* (Bezerra et al., 2002) activities and also showed to be an effective antioxidant agent (Paula-Junior et al., 2006).

The external mesocarp is the part that presents the biggest dimension in the pequi fruit but it is thrown away since this is a non edible part of the fruit. The disposal of the external mesocarp generates a huge amount of solid residues that could be used, adding value to the plant. In this work, the phytochemical study of this part of the fruit, together with the epicarp, is presented, since no systematic phytochemical study addressing these parts were found in the literature. Twelve compounds were isolated, pure or as constituents of mixtures. Pequi fruit ethanol extract, fractions and some of the isolated compounds were tested for their antioxidant activity using the radical DPPH (1,1-diphenyl-2-picrylhydrazyl)

spectrophotometric assay, as well as for their allelopathic activity, evaluating their effect on the growth of radicle and shoot of *Lactuca sativa* (lettuce).

## MATERIALS AND METHODS

### General Experimental Procedures

Melting points were determined with a Kofler hot plate apparatus and are uncorrected. Infrared (IR) spectra were recorded with a *Spectrum One* with ATR-IR, from Perkin Elmer. Nuclear Magnetic Resonance (NMR) spectra were recorded in CD<sub>3</sub>OD, D<sub>2</sub>O, CDCl<sub>3</sub> and C<sub>5</sub>D<sub>5</sub>N, at room temperature, on Bruker Avance DX-200 and DRX 400 MHz spectrometers. Absorbance, in DPPH assay, was measured in a Hitachi 2010 spectrophotometer. Silica gel Merck (Darmstadt, Germany) 100-200 and 200-425 mesh were used for column chromatography and silica gel Merck 60G was used for thin layer chromatography. Polyamide was purchased from Macherey Nagel (Düren, Germany). Solvents PA and HPLC grade were purchased from Vetec (Brazil) and Sigma Chemicals Co (St. Louis, USA), respectively. BHT (2,6-di-tert-butyl-4-methylphenol) and DPPH (1,1-diphenyl-2-picrylhydrazyl), were also purchased from Sigma.

### Plant Material

Fruits of *Caryocar brasiliense* were collected in January 2008, in Curvelo region, Minas Gerais, Brazil, and identified by Dr. João Renato Stehmann. A voucher specimen (No. 120826) was deposited at the Herbarium of the Natural History Museum of UFMG, Belo Horizonte, Minas Gerais, Brazil.

### Extraction and Isolation

The fruit was open and the internal mesocarp was discharged. The external mesocarp, together with the epicarp was grinded in ethanol, using a liquefier, and after 14 days at room temperature, the mixture was filtered through a cotton plug followed by Whatman filter paper. The extract was concentrated with a rotatory evaporator and 256.0 g of ethanol extract were obtained. A portion (130.0 g) of it was chromatographed over polyamide column (106.0 g), eluted with water, methanol and ethyl acetate pure and mixtures of decreasing polarities. Twenty-eight fractions of 100 mL each were collected, and reunited, by silica gel TLC, in five groups of fractions (G-1 to G-5). G-1 (72.6 g) was dissolved in water and extracted with diethyl ether; subsequent evaporation of

the solvents afforded 6.0 g of ethereal fraction (G-1E) and 66.6 g of insoluble residue (T1).

G-1E (6.0 g) was submitted to a silica gel column chromatography, with CH<sub>2</sub>Cl<sub>2</sub>, CH<sub>3</sub>OH and H<sub>2</sub>O, as eluent, either pure or in mixtures of increasing polarity: 48 fractions of 50 mL each were collected, and pooled in 11 groups of fractions. Group of fractions 2 (635.8 mg) was rechromatographed on silica gel column with hexane, CH<sub>2</sub>Cl<sub>2</sub> and ethyl acetate as eluent. A white solid precipitated from several fractions and, by washing with CH<sub>2</sub>Cl<sub>2</sub> and filtration, 1.4 g of a pure compound were obtained. This compound, pure by TLC, was identified as ethyl gallate (**1**). The solvents from the filtration were evaporated and the residue (150.6 mg) was rechromatographed on silica gel column: group of fractions 31-36 (41.0 mg) was found to be pure by TLC and was identified as 5-hydroxy-methylfurfural (**2**).

Group of fractions 6 (618.1 mg), from G-1E, was submitted to silica gel column chromatography (eluents: hexane, dichloromethane and ethyl acetate, either pure or in mixtures of increasing polarity). From group of fractions 2 (fractions 20-24), gallic acid (**3**) was isolated (201.0 mg). From group of fractions 8, from G-1E (594.6 mg), after silica gel column chromatography, 8.0 mg of pure methyl shikimate (**4**) were isolated.

Part of G-2 (9.5 g), from polyamide column, was chromatographed over silica gel column, with dichloromethane, ethyl acetate and methanol, as eluent. Eight groups of fractions were obtained and group of fractions 2 (fractions 16-19) furnished additional 1.03 g of ethyl gallate (**1**), by precipitation. Group of fractions 4 (fractions 25-28, 2.1 g), from G-2, after rechromatography over silica gel column, gave 146.0 mg of a mixture of β-D-fructopyranose (**5**) and β-D-fructofuranose (**6**). Group of fractions 5 (fractions 29-30, 2.3 g), from G-2, was also submitted to chromatography on silica gel and led to the isolation of a white solid (8.5 mg), that was identified as a mixture of α-D-glucose (**7**) and β-D-glucose (**8**). Part of G-3 (8.0 g), from polyamide column, after submitted to silica gel column chromatography, using the same eluent described, gave 9 groups of fractions. Group of fractions 1 (0.3 g) was rechromatographed on silica gel column (eluent hexane/acetone, either pure or in mixtures of increasing polarity), and 23 fractions were obtained. From fractions 1-7 (0.27 g), 8.0 mg of lupeol (**9**) and oleic acid (**10**) mixture and 20.0 mg of β-sitosterol (**11**), stigmasterol (**12**) and

oleic acid (**10**) mixture were isolated. From fractions 7-18, 1.15 g of ethyl gallate (**1**) were obtained by filtration.

### Spectrometric data

**Compound 1** (Ethyl gallate): 3.58 g, 14.0 % yield. white solid, m.p.: 170.2-172.1 °C. IR (KBr, cm<sup>-1</sup>): 3446, 3290, 2973, 2933, 1704, 1615, 1533, 1469, 1309 and 1250. <sup>1</sup>H NMR (400 MHz, C<sub>5</sub>D<sub>5</sub>N) δ<sub>H</sub> (H, m, *J* in Hz): 7.9 (H-2), 7.9 (H-6), 4.3 (H-8, q, 7.2); 1.2 (H-9, t, 7.2). <sup>13</sup>C NMR (100 MHz, C<sub>5</sub>D<sub>5</sub>N) δ<sub>C</sub>: 121.8 (C-1); 110.6 (C-2); 148.0 (C-3); 141.3 (C-4); 148.0 (C-5); 110.6 (C-6); 167.5 (C-7); 60.8 (C-8); 14.8 (C-9).

**Compound 2** (5-Hydroxymethylfurfural): 41.0 mg, 0.02 % yield, viscous oil. IR (KBr, cm<sup>-1</sup>): 3369, 1658, 1582, 1519 and 1018. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> (H, m, *J* in Hz): 7.2 (H-3, d, 3.4); 6.5 (H-4, d, 3.4); 9.6 (H-6, s); 4.7 (H-7, s); 2.7 (s, OH). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ<sub>C</sub>: 152.3 (C-2); 123.0 (C-3); 110.0 (C-4); 160.8 (C-5); 177.8 (C-6); 57.6 (C-7).

**Compound 3** (Gallic acid). 201.0 mg, 0.08 % yield. white solid, m.p.: 245-248 °C. IR (KBr, cm<sup>-1</sup>): 3500, 3450-2600, 1650, 1600, 1540 and 1350. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ<sub>H</sub> (H, m): 7.1 (H-2, H-6, s); 9.0 (OH, s). <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ<sub>C</sub>: 122.1 (C-1); 110.5 (C-2 and C-6); 146.5 (C-3 and C-5); 139.7 (C-4); 170.6 (C-7);

**Compound 4**: Methyl shikimate (8.0 mg, 0.003 % yield): white solid, m.p.: 108-109 °C. IR (KBr, cm<sup>-1</sup>): 3303, 1715, 1657 and 1233. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ<sub>H</sub> (H, m, *J* in Hz): 6.8 (H-2, m); 4.4 (H-3, br s); 3.7 (H-4, dd, 6.8 and 3.6); 4.0 (H-5, m); 2.2 and 2.7 (H-6, m); 4.8 (H-8, s); 4.5 (OH, br s). <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ<sub>C</sub>: 130.0 (C-1); 139.3 (C-2); 67.4 (C-3); 72.7 (C-4); 68.6 (C-5); 31.6 (C-6); 168.9 (C-7); 52.5 (C-8).

**Mixture 1** [β-D-fructopyranose (**5**) and β-D-fructofuranose (**6**): 146 mg, 0.06 % yield. β-D-Fructopyranose (**5**). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ<sub>H</sub> (H, m): 3.5 and 3.7 (H-1, m); 3.8 (H-3, br s); 3.9 (H-4, br s); 4.0 (H-5, d); 3.7 and 4.0 (H-6, m). <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ<sub>C</sub>: 64.7 (C-1); 99.3 (C-2); 69.6 (C-3); 72.0 (C-4); 71.4 (C-5); 64.7 (C-6). β-D-fructofuranose (**6**). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ<sub>H</sub> (H, m): 3.6 and 3.7 (H-1, m); 4.0-4.1 (H-3, H-4 and H-5, m); 3.5 and 3.8 (H-6, m). <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ<sub>C</sub>: 64.4 (C-1); 103.3 (C-2); 77.7 (C-3); 77.0 (C-4); 83.5 (C-5); 64.4 (C-6).

**Mixture 2** [α-D-glucose (**7**) and β-D-glucose (**8**): 8.5 mg, 0.0033 % yield. α-D-Glucose (**7**). <sup>1</sup>H NMR (400

MHz, D<sub>2</sub>O)  $\delta_{\text{H}}$  (H, m, *J* in Hz): 5.2 (H-1, d, 3.6); 3,4 (H-2, m); 3.7 (H-3, t, 9.6); 3.4 (H-4, t, 9.6); 3.7–3.8 (H-5, m); 3.7 (H-6, dd, 5.6 and 12.4). <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O)  $\delta_{\text{C}}$ : 92.1 (C-1); 71.5 (C-2); 72.8 (C-3); 69.6 (C-4); 71.3 (C-5); 60.6 (C-6).  $\beta$ -D-Glucose (**8**). <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O)  $\delta_{\text{H}}$  (H, m, *J* in Hz): 4.6 (H-1, d, 8.2); 3.2 (H-2, t, 8.8); 3.4–3.5 (H-3, H-4, H-5, m); 3.7–3.8 (H-6, m). <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O)  $\delta_{\text{C}}$ : 95.9 (C-1); 74.1 (C-2); 75.7 (C-3); 69.6 (C-4); 75.9 (C-5); 60.7 (C-6).

**Mixture 3** [lupeol (**9**) and oleic acid (**10**): 8.0 mg, 0.003 % yield: Lupeol (**9**). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta_{\text{C}}$ : 38.4 (C-1); 27.5 (C-2); 79.4 (C-3); 39.0 (C-4); 55.6 (C-5); 18.7 (C-6); 34.6 (C-7); 41.2 (C-8); 50.8 (C-9); 37.5 (C-10); 21.3 (C-11); 25.0 (C-12); 38.4 (C-13); 43.2 (C-14); 27.5 (C-15); 35.9 (C-16); 43.3 (C-17); 48.3 (C-18, C-19); 151.3 (C-20); 29.4 (C-21); 40.3 (C-22); 28.3 (C-23); 15.7 (C-24); 16.4 (C-25); 16.3 (C-26); 14.9 (C-27); 18.2 (C-28); 109.6 (C-29); 19.6 (C-30). Oleic acid (**10**) <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 178.7 (C-1); 34.1 (C-2); 25.0 (C-3); 29.6 (C-4); 29.4 (C-5); 29.5 (C-6); 29.6 (C-7); 27.8 (C-8); 130.3 (C-9); 130.1 (C-10); 27.7 (C-11); 29.7 (C-12); 29.9 (C-13); 29.8 (C-14 and C-15); 32.2 (C-16); 23.0 (C-17); 14.4 (C-18).

**Mixture 4** [ $\beta$ -sitosterol (**11**), stigmasterol (**12**) and oleic acid (**10**): 20.0 mg, 0.0075 % yield.  $\beta$ -Sitosterol (**11**) and Stigmasterol (**12**). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta_{\text{C}}$ : 37.3 (C-1); 31.7 (C-2); 71.9 (C-3); 42.3 (C-4); 140.8 (C-5); 121.8 (C-6); 32.0 (C-7); 31.9 (C-8); 50.2 (C-9); 36.2 (C-10); 21.1 (C-11); 39.7 (C-12); 42.3 (C-13); 56.9 (C-14); 24.4 (C-15); 28.9 (C-16); 56.0 (C-17); 12.1 (C-18); 19.4 (C-19); 36.2 (C-20 for  $\beta$ -sitosterol); 40.5 (C-20 for stigmasterol); 19.0 (C-21 for  $\beta$ -sitosterol); 21.2 (C-21 for stigmasterol); 34.0 (C-22 for  $\beta$ -sitosterol); 138.3 (C-22 for stigmasterol); 26.1 (C-23 for  $\beta$ -sitosterol); 129.8 (C-23 for stigmasterol); 45.9 (C-24 for  $\beta$ -sitosterol); 51.3 (C-24 for stigmasterol); 29.2 (C-25 for  $\beta$ -sitosterol); 31.9 (C-25 for stigmasterol); 19.0 (C-26); 19.8 (C-27 for  $\beta$ -sitosterol); 19.0 (C-27 for stigmasterol); 22.7 (C-28 for  $\beta$ -sitosterol); 25.4 (C-28 for stigmasterol); 12.0 (C-29 for  $\beta$ -sitosterol); 12.3 (C-29 for stigmasterol).

### DPPH Radical Scavenging Assay

Radical scavenging activities of extracts and flavonoids (Table 1) were determined according to the method described by Burda and Oleszek (2001). BHT (2,6-di-tert-butyl-4-methylphenol) was used as

reference compound. Samples and BHT (750.0  $\mu$ L) were prepared in triplicate for each concentration used (1.0, 10.0 and 100.0  $\mu$ g/mL) and, to each flask, the volume was adjusted to 2.0 mL by adding 1.5 mL of a 0.002 % p/v solution of DPPH in methanol. The solutions were shaken vigorously and kept in the dark for 30 min. The control was prepared as above without any extract or substance. Absorbance (measured on a Hitachi 2101 spectrophotometer) was measured at 517 nm and methanol was used for the baseline correction.

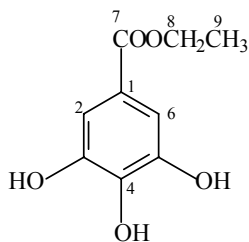
Radical scavenging activity was expressed as the inhibition percentage and was calculated:

$$\left\{ \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right\} \times 100$$

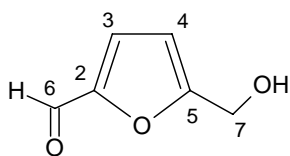
where  $\text{Abs}_{\text{control}}$  = absorbance of DPPH radical in methanol and  $\text{Abs}_{\text{sample}}$  = absorbance of the extracts and pure substances in methanol + DPPH. Scavenging activities were expressed in  $\mu$ g/mL. IC<sub>50</sub> values ( $\mu$ g/mL) was expressed the concentration of sample necessary to scavenge 50% of DPPH free radicals.

### Allelopathic Bioassay

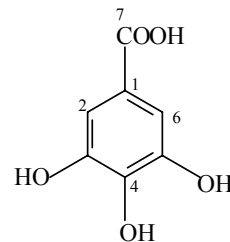
*Lactuca sativa* (cv Grand Rapids) seeds were purchased from Isla Pak, RS, Brazil. All undersized and damaged seeds were discarded. According to methodology described by Vieira et al. (2005), germination and growth were conducted in 100 mm Petri dishes containing 9.0 cm sheet of Whatman no. 1 filter paper as suport. Then, 25 lettuce seeds were placed per dish with 10 mL of a test ( $10^{-4}$ ,  $10^{-6}$  and  $10^{-8}$  M) or a control solution (without substance). All solutions were prepared with deionized water and their pH values [buffered with 10 mM 2-(*N*-morpholino) ethanesulfonic acid, MES] were adjusted to 6.0 - 6.5 with NaOH solution. Concentrations lower than  $10^{-4}$  M were obtained by dilution series. All tests were triplicated. Dishes were covered with Parafilm to reduce evaporation and incubated in the dark at 25 °C, in a controlled-environment growth chamber, for 5 days. After this time, the number of germinated seeds were counted (a seed was considered to be germinated when the radicle was at least 0.2 mm long), the lengths of radicle and shoots were measured (using a paquimeter). During the measurement process, the dishes were kept at 4 °C to avoid subsequent growth. The osmotic pressure values were measured on a microsmometer and ranged between 30 and 38 mOsmolar.



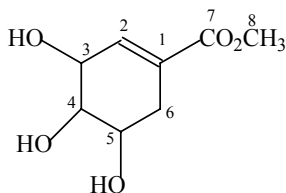
Ethyl galate (1)



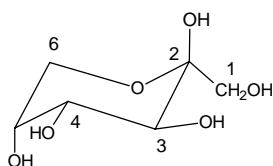
5-Hydroxymethylfurfural (2)



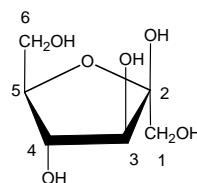
Galic acid (3)



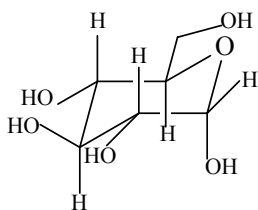
Methyl shikimate (4)



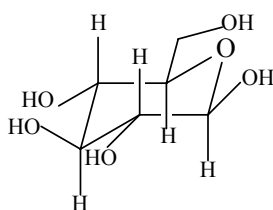
β-D-Frutopyranose (5)



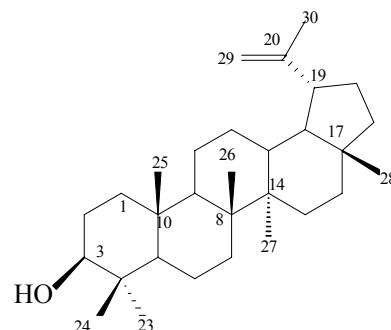
β-D-Fructofuranose (6)



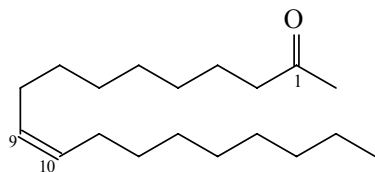
α-D-Glucose (7)



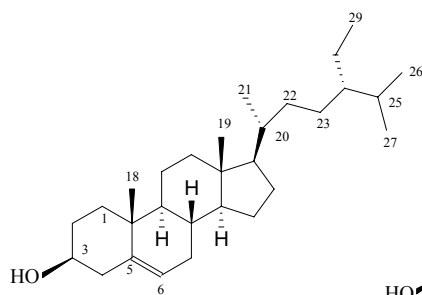
β-D-Glucose (8)



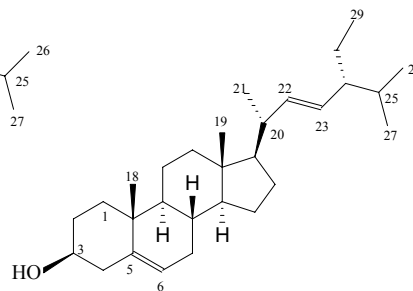
Lupeol (9)



Oleic acid (10)



β-Sitosterol (11)



Stigmasterol (12)

**Table 1** – Antioxidant activity of ethanol extract and fractions from epicarp and external mesocarp of *C. brasiliense* Camb. (pequi), determined by DPPH method, in three different concentrations.

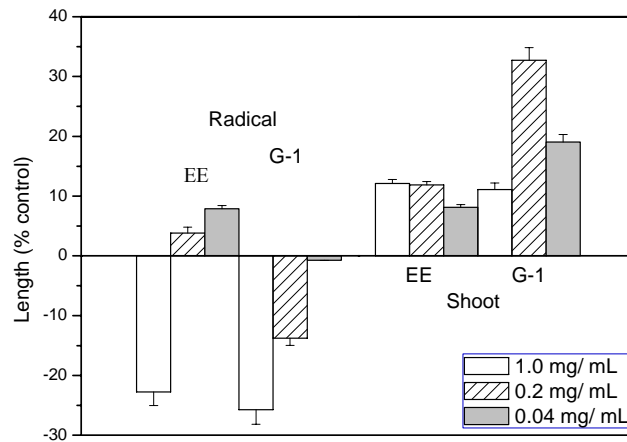
Extracts /fractions	(1 µg/mL)	Inhibition (%)		IC <sub>50</sub> (µg/mL)
		(10 µg/mL)	(100 µg/mL)	
EE	0.0	63.7	94.4	28.3±6.6
G-1	5.4	51.3	96.7	9.3±6.3
G -1E	16.6	95.5	96.9	2,4±0.8
T1	3.6	16.9	91.7	22.2±2.5
BHT	40.4	45.6	84.2	16.4 ±3.6

**Table 2** – Antimicrobial activity, *in vitro*, of G-1E fraction, obtained from epicarp and external mesocarp of *C. brasiliense* Camb. (pequi) towards several bacterial strain and the yeast *C. albicans*

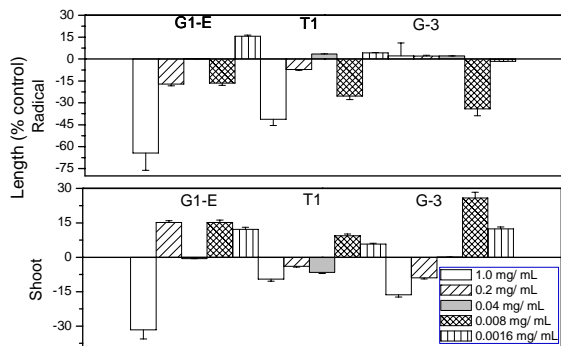
Inhibition zones (mm) <sup>a</sup>			
Microrganisms	G -1E	Chloranfenicol	Miconazol
<i>S. aureus</i>	13.0±2.8	20±2.8	nt
<i>S. typhymurium</i>	11.0±1.41	20±0.0	nt
<i>E. coli</i>	12.0±2.8	23±1.4	nt
<i>C. freundi</i>	11.0±0.0	22±0.7	nt
<i>B. cereus</i>	11.0±2.8	24±1.4	nt
<i>L. monocytogenes</i>	18.0±2.8	30±1.4	nt
<i>P. aeruginosa</i>	10.0±1.4	14±2.1	nt
<i>C. albicans</i>	0	nt	25±2.1

nt = not tested; <sup>a</sup>Values are mean ± SD of triplicate determinations.

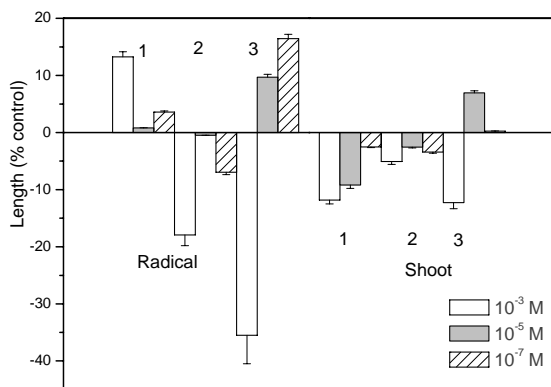
**Figure 2.** Effect of ethanol extract (EE) and G-1 group from epicarp and external mesocarp of *C. brasiliense* Camb. (pequi) on radical and shoot length of *L. sativa*, in three different concentrations. Values are presented as percentage differences from the control, zero representing an observed value identical to the control (solution without substance), a positive value representing stimulation and a negative value representing inhibition.



**Figure 3.** Effect of ethereal fraction (G1-E), ethereal insoluble fraction (T1) and G-3, from epicarp and external mesocarp of *C. brasiliense* Camb. (pequi) on radical and shoot length of *L. sativa*, in three different concentrations. Values are presented as percentage differences from the control (solution without substance), zero representing an observed value identical to the control, a positive value representing stimulation and a negative value representing inhibition.



**Figure 4.** Effect of ethyl galate (1), hydroxymethylfurfural (2) and galic acid (3), isolated from epicarp and external mesocarp of *C. brasiliense* Camb. (pequi) on radical and shoot length of *L. sativa*, in three different concentrations. Values are presented as percentage differences from the control (solution without substance), zero representing an observed value identical to the control, a positive value representing stimulation and a negative value representing inhibition.



**Data Analysis**

The effect on germination and growth are given as percent differences from control, and consist of the differences (in cm) between mean values of seeds with tested compounds and mean values for control (seeds grown without addition of tested compounds)/ mean values for control x 100. Thus, zero represents the control, positive values represent stimulation of the studied parameter and negative values represent inhibition. The data were evaluated

by using Student’s *t*-test and the differences between the experiment and control were significant at a value of  $P \leq 0.05$ . The inhibitory and stimulatory activities, compared to those of the control, are shown in Figures 2, 3 and 4.

**Antimicrobial Bioassay**

Samples were tested in duplicate by disc diffusion method in agar with minor modifications (Lana et al., 2003). Microorganisms used were *Staphylococcus aureus* ATCC 29212, *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 25922, *Citrobacter freundii* ATCC 8090, *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* ATCC 15313, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 18804. For minimum inhibitory concentration test, carried out in duplicate, samples were serially diluted starting from concentration of 512 to 5.0 µg/ml for each test microorganism. Tubes were incubated for 18 hours at 35°C. The results are shown in Table 2

**RESULTS AND DISCUSSION**

A total of twelve compounds were isolated from the pequi fruit epicarp and external mesocarp parts. Their structures were determined based on the analysis of spectroscopic data, especially NMR, and literature data comparison. The pure compounds were identified as ethyl gallate (1) (Ceruks et al., 2007), 5-hydroxymethylfurfural (2) (Kuo et al., 2002), gallic acid (3) (Souza Filho et al., 2006) and methyl shikimate (4) (Liu et al., 2004; Adrio et al., 1997). Additionally, mixtures containing β-D-fructopyranose (5) and β-D-fructofuranose (6) (Sobolev et al., 2003; Breitmaier and Voelter, 1987); α- and β-D-glucose (7 and 8) (Collins and Ferrier, 1995); lupeol (9) (Mahato and Kundun, 1994) and oleic acid (10) (Oliveira et al., 2006); β-sitosterol (11), stigmasterol (12) (Goulart et al., 1993) and oleic acid (10) were obtained. The structures of these isolated compounds were assigned on the basis of spectroscopic data, including two-dimensional NMR methods and by comparison of their spectral data with values described in the literature. Structures of compounds 1-12 can be found in Figure 1.

Ethyl gallate (1) was isolated on a very high yield (14% from the crude ethanol extract, EE), whereas compounds 2, 3 and 4 were isolated on a very low yield from EE.

Ethyl gallate and gallic acid were previously isolated from leaves of *Caryocar microcarpum* (Kawanishi and Raffaud, 1986). Oleic acid,  $\beta$ -sitosterol and stigmasterol have already been isolated from *C. microcarpum* and *C. villosum* (Kawanishi and Raffaud, 1986; Marx et al., 1997).

Crude ethanol extract (EE), fractions G-1 obtained from polyamide column, G-1E (fraction soluble in ethyl ether, obtained from G-1) and T1 (ethyl ether insoluble residue, obtained from G-1) were evaluated for their antioxidant activities (Burda and Oleszek, 2001). Table 1 shows the average values for DPPH radical scavenging activity in each tested concentration, and IC<sub>50</sub> values. G1-E presented the higher IC<sub>50</sub>, that can be associated to the presence of gallic acid and ethyl gallate in higher concentrations than in EE and T1. At 100  $\mu$ g/mL, all extracts tested were more active than BHT, the reference compound.

The obtained results from allelopathic evaluation, according to methodology described by Vieira et al. (2005), of for crude ethanol extract, fractions and compounds **1**, **2** and **3** are shown in Figures 2, 3 and 4. Both EE and G-1 showed inhibitory activity on radical growth and stimulatory activity on shoot growth of *L. sativa*. G-1 presented biggest inhibitory effect at 1.0 mg/mL, and also biggest stimulatory effect at 0.2 mg/mL (Figure 2). Fractions G-1E, T1 e G-3 (Figure 3) were tested at concentrations below 0.04 mg/mL, aimed to observe more stimulation on shoot and radical growth (Macías et al., 2000). However, only G1-E showed a slight stimulatory effect on radical growth at  $1.6 \times 10^{-3}$  mg/mL and the inhibitory effects were bigger than those presented for EE and G-1, at analogous concentrations. On shoot, the best growth stimulatory effect was observed for G-3, at  $8.0 \times 10^{-3}$  mg/mL. The effect of pure compounds **1**, **2** and **3** on radicle and shoot growth of *L. sativa* was mainly inhibitory (Figure 4). Gallic acid (**3**) presented the biggest inhibitory effect on radicle, at  $10^{-3}$  M, and also the biggest stimulatory effect on shoot, at  $10^{-7}$  M.

Crude ethanol extract (EB) and fractions G-1 and G-1E were tested for their antimicrobial activity (Lana et al., 2003), presenting inhibition zones of 7 mm. Only fraction G-1E presented expressive activity towards the tested microorganisms, according to Table 2. *C. albicans* was not affected by this fraction in the tested concentration. The minimum inhibitory concentration (MIC) found for

all tested microorganisms, except for *S. typhimurium* and *C. albicans* was 512  $\mu$ g/mL.

## CONCLUSIONS

This work pointed out for the possibility to use the external mesocarp of pequi fruit as a rich source of ethyl gallate, since this compound was isolated in an expressive yield (14 % from crude ethanol extract). The biological potential of this crude extract and isolated compounds were also noticeable (high IC<sub>50</sub> in the antioxidant evaluation of extracts, and both inhibitory and stimulatory effect on radical and shoot growth of *L. sativa*, respectively from gallic acid), since plant material used is a residue produced in large scale in Brazil.

## ACKNOWLEDGEMENTS

To CNPq, for JAT e MADB grants. To FAPEMIG, for financial help.

## REFERENCES

- Adrio J, Carretero JC, Ruano JLG, Cabrejas LMM. 1997. Enantioselective synthesis of (+)-shikimic acid and (+)-5-epi-shikimic acid by asymmetric Diels-Alder reaction of (+)- $\alpha$ -sulfinylacrylates. *Tetrahedron Assym.* 8:1623-1631.
- Azevedo MCH, Rodriguez ADB. 2004. Confirmation of the identity of the carotenoids of tropical fruits by HPLC-DAD and HPLC-MS. *J. Food Compos. Anal.* 17:385-396.
- Bezerra JCB, Silva IA, Ferreira HD, Ferri PH, Santos SC. 2002. Molluscicidal activity against *Biomphalaria glabrata* of brazilian cerrado medicinal plants. *Fitoterapia* 73:428-430.
- Breitmaier E, Voelter W. 1987. Carbon 13 NMR spectroscopy high-resolution methods and applications in organic chemistry and biochemistry. 3<sup>th</sup> ed., Weinheim: New York, 515 p.
- Burda S, Oleszek PM. 2001. Antioxidant and antiradical activities of flavonoids. *J. Agric. Food Chem.* 49:2774-2779.
- Ceruks M, Romoff P, Fávero AO, Lago, JHG. 2007. Constituintes fenólicos polares de *Schinus terebinthifolius* Raddi (Anacardiaceae). *Quim. Nova* 30:597-599.
- Collins P, Ferrier, R. 1995. Monosaccharides, their chemistry and their roles in natural products; 1<sup>st</sup> ed, John Wiley & Sons: Chichester, 1995, 564 p.
- Damiani, C. Qualidade e perfil volátil de pequi (*Caryocar brasiliense* Camb.) minimamente processado, armazenado sob diferentes temperaturas. 2006. MSc Thesis. Universidade Federal de Lavras. Brasil. 127p.

- Goulart MOI, Sant'Ana AEG, Lima RA, Cavalcante SH. 1993. Fitoconstituintes químicos isolados de *Jatropha elliptica*. Atribuição dos deslocamentos químicos dos átomos de carbono e hidrogênio dos triterpenos de jatrolonas A e B. *Quim. Nova* 16:95-100.
- Herzog-Soares JD, Alves RK, Isac E, Bezerra JCB, Gomes MH, Santos SC, Ferri PH. 2002. Atividade tripanocida in vivo de *Stryphnodendron adstringens* (barbatimão verdadeiro) e *Caryocar brasiliense* (pequi). *Rev Bras. Farmacogn.* 12:1-2.
- Kawanishi K, Raffaud RF. 1986. *Caryocar microcarpum*: an ant repellent and fish poison of the nortweat amazon. *J. Nat. Prod.* 49:1167-1168.
- Kuo Y, Lee P, Wein Y. 2002. Four new compounds of seeds of *Cassia fistula*. *J. Nat. Prod.* 65:1165-1167.
- Lana E JL, Carazza F, Takahashi JA. 2003. Antibacterial evaluation of some new 2-aryl-3,5-dimethoxy-1,4-benzoquinone derivatives. *J. Agric. Food Chem.* 54:2053-2056.
- Liu A, Liu ZZ, Zou ZM, Chen SZ, Xu LZ, Yang SL. 2004. Synthesis of (+)-zeylenone from shikimic acid. *Tetrahedron* 60:3689-3694.
- Macías FA, Castellano D, Molinillo JMG. 2000. Search for standard phytotoxic bioassay for allelochemicals. Selection for standard target species. *J. Agric. Food Chem.* 48:2512-2521.
- Magalhães HG, Monteiro Neto H, Lagrota MH, Wigg MD, Guimarães LAS, Loja MASO, Araújo RR. 1988. Estudo estrutural do pequi *Caryocar brasiliense* Camb. Caryocaraceae, sob o aspecto farmacológico e botânico. *Rev. Bras. Farm.* 69:31-41.
- Mahato SB, Kundun AP. 1994. <sup>13</sup>C NMR spectra of pentacyclic triterpenoids—a compilation and some salient features. *Phytochemistry* 37:1517-1575.
- Marx F, Andrade EHA, Maia JG. 1997. Chemical composition of the fruit pulp of *Caryocar villosum*. *Zeitsch. Lebensm. Unters.Forsch.* 204:442-444.
- Oliveira DM, Silva GDF, Duarte LP, Vieira Filho SA. 2006. Chemical constituents isolated from roots of *Maytenus acanthophylla* Reissek (Celastraceae). *Biochem. Syst. Ecol.* 34:661-665.
- Passos XS, Santos SC, Ferri PH, Fernandes OF, Paula TF, Garcia AC, Silva MR. 2002. Antifungal activity of *Caryocar brasiliense* (Caryocaraceae) against *Cryptococcus neoformans*. *Rev. Soc. Bras. Med. Trop.* 35:623-627.
- Paula-Junior W, Rocha FH, Donatti L, Fadel-Picheth CMT, Weffrt-Santos AM. 2006. Leishmanicidal, antibacterial, and antioxidant activities of *Caryocar brasiliense* Cambess leaves hydroethanolic extract. *Rev. Bras. Farmacogn.* 16:625-630.
- Prance GT. 1990. The genus *Caryocar* L. (Caryocaraceae): an underexploited tropical resource. *Adv. Econ. Bot.* 8:177-188.
- Sobolev AP, Segre A, Lamanna R. 2003. Proton high-field NMR study of tomato juice. *Magn. Reson. Chem.* 41:237-245.
- Souza Filho APS, Santos RA, Santos LS, Guilhon GMP, Santos AS, Arruda MSP, Muller AH, Arruda AC. 2006. Potencial alelopático de *Myrcia guianensis*. *Planta Daninha* 24:649-652.
- Vieira RF, Martins MVM. 2000. Recursos genéticos de plantas medicinais de cerrado: uma compilação de dados. *Braz. J. Med. Plant.* 3:13-36.
- Vieira HS, Takahashi JA, Pimenta LPS, Boaventura MADZ. 2005. Effects of kaurane diterpene derivatives on germination and growth of *Lactuca sativa* seedlings. *Z. Naturf. C* 60:72-78.

