Anais da Academia Brasileira de Ciências (2019) 91(2): e20180248
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-3765201920180248
www.scielo.br/aabc | www.fb.com/aabcjournal

Jabuticaba (*Plinia jaboticaba*) skin extracts as inhibitors of phospholipases A_2 and proteases

TAMARA R. MARQUES, MARIANA A. BRAGA, PEDRO H.S. CESAR, SILVANA MARCUSSI and ANGELITA D. CORRÊA

Departamento de Química, Universidade Federal de Lavras/UFLA, Campus Universitário, Caixa Postal 3037, 37200-000 Lavras, MG, Brazil

Manuscript received on March 3, 2018; accepted for publication on August 8, 2018

How to cite: MARQUES TR, BRAGA MA, CESAR PHS, MARCUSSI S AND CORRÊA AD. 2019. Jabuticaba (*Plinia jaboticaba*) skin extracts as inhibitors of phospholipases A_2 and proteases. An Acad Bras Cienc 91: e20180248. DOI 10.1590/0001-3765201920180248.

Abstract: The phenolic extracts of jabuticaba skin flour (JSF) were characterized by HPLC, and evaluated for their modulating action upon phospholipases A_2 and proteases of snake venom, aiming at their possible use in the treatment of the various diseases associated with the action of venom toxins. Two types of extracts were prepared from JSF: aqueous and methanolic. These extracts, evaluated at different ratios, (venom: extract, m/m), significantly inhibited the phospholipase activity induced by the venom of *Bothrops moojeni* and *Crotalus durissus terrificus*, except for *Bothrops atrox* venom. The greatest hemolysis inhibitory action was observed for the methanolic extract, when incubated with venoms of *B. moojeni* and *C. durissus terrificus*, with inhibitions between 21 and 100%. Thrombolysis induced by venoms of *B. moojeni* and *C. durissus terrificus* was inhibited by both extracts, ranging from 32 to 83% and 51 to 83% for the aqueous and methanolic extracts, respectively. Both extracts extended coagulation time, induced by the venoms of *B. moojeni* and *Lachesis muta muta*. Inhibitory actions are related to phenolic compounds, such as gallic, syringic and *p*-coumaric acids, besides catechin, epigallocatechin gallate, epicatechin; resveratrol and quercetin, present in the extracts of jabuticaba skin flour, confirming their potential for nutraceutical use.

Key words: agro-industrial waste, phenolic compounds, venom enzymes as laboratory tools, clotting, thrombolysis.

INTRODUCTION

The jabuticaba tree is a plant native to Brazil, belonging to the family *Myrtaceae*, and it is found in a wide territorial area of the country, from the State of Pará to Rio Grande do Sul, but it is in the states of the Southeast that the highest production occurs. Among the most appreciated species, *Plinia jaboticaba* (Vell.) Berg, popularly known as 'Jabuticaba Sabará', stands out (Citadin et al. 2010).

Correspondence to: Dr. Silvana Marcussi
E-mail: marcussi@dqi.ufla.br
ORCid: https://orcid.org/0000-0002-4674-6911

The fruit is rich in phenolic compounds, mainly in its skin, with contents ranging from 9.79 to 11.99 g 100 g^{-1} dry matter, in addition to vitamin C, minerals and fibers (Lima et al. 2008, Alves et al. 2013, 2014), and it has attracted the attention of researchers, being used for various medicinal purposes, such as in the treatment of diarrhea and skin irritation, as well as indications in popular medicine against asthma and other diseases (Ascheri et al. 2006, Sato and Cunha 2009). The fruit is frequently used in the production of sweets, jellies, juices and alcoholic beverages, which generates large amounts of residues, mainly represented by the skins (Fortes et al. 2011, Abe et al. 2012).

In the skin, which is generally discarded and may represent up to 43% of the fruit (Lima et al. 2008), the highest levels of nutrients and phytochemicals that are beneficial to health are found. Studies have reported the use of skin as dyes in yoghurts (Alves et al. 2013) and in the preparation of restructured hams (Alves et al. 2017), making these products rich in fibers and phenolic compounds with antioxidant activity; as well as the use in humans and rats to reduce serum levels of oxidative peroxidation and increased antioxidant defense (Lage et al. 2014, Lenquiste et al. 2015, Batista et al. 2016). Therefore, its chemical and pharmacological characterization is of great economic interest, adding value to the residue and directing to its possible applications, mainly in the development and manufacture of functional and nutraceutical products that might be used in health improvement, in the maintenance of well-being, in improving immunity and, therefore, in the prevention, as well as in the treatment, of various diseases.

Phenolic compounds have potential for the production of nutraceuticals and functional foods, due to their antioxidant capacity, specific interaction with several enzymes and protective role on membranes and modulators on the immune response (Prakask et al. 2012, Kumar 2015, Peluso and Serafini 2017). Studies report their action in the prevention of various diseases, such as cancer, atherosclerosis, menopausal symptoms and degenerative diseases such as Parkinson's, multiple sclerosis and Alzheimer's (Babbar et al. 2015, Kumar and Kumar 2015, Olaiya et al. 2016, Evans et al. 2016, Wong and Evans 2017). They are also responsible for inhibiting toxic and/or pharmacological effects induced by snake venoms, acting as enzymatic inhibitors (Santhosh et al. 2013, Nanjaraj et al. 2013).

The snake venoms contain proteases and phospholipases A_2 that can act on several components of the coagulation cascade, receptors and components of the extracellular matrix and of the basement membrane, as well as on components of platelet membranes. These enzymes can display up to 96% of homology with their human homologues (Sajevic et al. 2011, Trusevych and MacNaughton 2015, Pozzi et al. 2016). Because of this high degree of structural and functional homology these can be used to induce the effects on hemostasis and enzymatic activities of phospholipases A_2 and proteases. Thus, the inhibition of phospholipases A_2 and some proteases present in snake venoms by natural compounds is believed to occur in a similar fashion upon animal enzymes homologues.

Phospholipases A_2 are considered as important regulators of the arachidonic acid pathway and can act as essential mediators of intracellular and intercellular signaling, hydrolyzing phospholipids and generating bioactive lipid mediators or messengers that control multiple cell functions and regulate physiological and pathophysiological processes (Ramanadham et al. 2004, Masuda et al. 2005, Cechetti et al. 2017). While the proteases act selectively in the factors of the coagulation cascade, with effect on platelet aggregation, fibrinolysis and coagulation (Braud and Wisner 2000).

In this context, jabuticaba skin could be used in the pharmaceutical, cosmetic and/or food industry, mainly due to its high levels of phenolic compounds that provide nutraceutical properties. Thus, in this study, we evaluated the toxicity and pharmacological characterization of the aqueous and methanolic extracts of jabuticaba skin flour upon hemostasis and enzymatic activities of phospholipases A_2 and proteases. To induce such effects, tests were carried out using snake venoms as tools aiming to extend the knowledge on the nutraceutical potential this jabuticaba skin waste.

MATERIALS AND METHODS

OBTENTION AND PREPARATION OF THE JABUTICABA SKIN FLOUR

Jabuticaba (*Plinia jaboticaba* (Vell.) Berg fruits (16 kg), were picked on São José do Ismeril Farm, in the

municipality of Coqueiral, MG, Brazil. The fruits were identified and incorporated into the collection of the ESAL Herbarium in the Department of Biology of the Federal University of Lavras - MG, under the registration number 19574.

They were selected, washed in tap water and then sanitized with sodium hypochlorite solution (200 mg kg^{-1}), by immersion for 10 minutes. Subsequently, the fruits were pressed, and 3.91 kg skins were obtained. The skins were placed in fine mesh metal baskets and dehydrated in a drying oven at 45 °C until constant weight.

After drying, the skins were ground in a knife mill (TE 631 Tecnal) for 3 minutes, and 0.82 kg jabuticaba skin flour (JSF) was obtained. The JSF passed through sieves, resulting in particle sizes between 40 mesh (0.425 mm) and 80 mesh (0.180 mm). Subsequently, the moisture content of this flour was determined in triplicate (AOAC 2016) and it was stored in hermetically sealed flasks, protected from light, at room temperature until its use.

PREPARATION OF THE JABUTICABA SKIN FLOUR EXTRACT

For the preparation of the JSF extracts, water and methanol were used to obtain two extracts, aqueous and methanolic, respectively. The extractions were performed in triplicate.

The aqueous extract was obtained using 1 g JSF mixed with 50 mL of distilled water, stirred for 15 minutes at room temperature, and filtered through organza cloth. The residue was subjected, twice more, to the same extraction process. The filtrates were collected, frozen and lyophilized. For the obtention of the methanolic extract, 1 g JSF was transferred to a 250 mL Erlenmeyer flask, mixed with 50 mL of 50% (v/v) methanol, capped with an appropriate stopper for reflux and subjected to a temperature of 80 °C on a hot plate, for 15 minutes. The extract was then filtered on filter paper and the residue was subjected to two extractions (AOAC 2016). The filtrates were collected, evaporated on a plate at 80 °C until complete elimination of methanol and then frozen and lyophilized.

PHYTOCHEMICAL SCREENING

The extracts of JSF passed through phytochemical screening process, using specific reagents for each chemical group, and applying chemical reactions that resulted in coloring and/or precipitation processes particular for each substances class (Matos 1997). The substances analyzed were tannins, flavonoids, catechins, saponins, cardiac glycosides, steroids and alkaloids.

a) Tannins: 1 mg of extracts was dissolved in 10 mL of distilled water and a drop of 1% ferric chloride was added. The change in color or formation of a precipitate indicated a positive reaction.
b) Flavonoids: 1 mg of extracts was dissolved in 10 mL of methanol. The mixture was filtered, and five drops of concentrated HCl and a one-cm piece of magnesium tape were added. A pink tint in the solution indicated a positive reaction.
c) Catechins: 1 mg of extracts was dissolved in 3 mL of methanol, were subsequently added 1 mL of 1% vanillin aqueous solution and 1 mL of concentrated HCl. The appearance of an intense red color indicated a positive reaction.
d) Saponins: 1 mg of the extracts was dissolved in 1 mL of 80° GL ethanol and was added 15 mL of distilled water. The solution was stirred and foamed for more than half an hour, indicated a positive reaction.
e) Cardiac glycosides: 1 mg of the extracts was solubilized in 4 mL of methanol and a drop of the Kedde reagent was added. The formation of a blue or violet color indicated a positive reaction.
f) Steroids: 1 mg of the extracts was solubilized in 3 mL of chloroform, and then 2 mL of

acetic anhydride was added and stirred gently. On the walls of the tube was added 1 mL of concentrated sulfuric acid. The formation of a succession of colors, from evanescent blue to persistent green indicated a positive reaction.

g) Alkaloids: 1 mg of the extracts was solubilized in 3 mL of 5% hydrochloric acid and was added 5 drops of the Bouchardat reagent. The formation of an orange-red precipitate indicated a positive reaction.

DETERMINATION OF THE PHENOLIC COMPOSITION OF THE EXTRACTS BY HPLC

Chromatographic analysis was performed with a Shimadzu high performance liquid chromatography system, equipped with two LC-20AT model high pressure pumps, an SPD-M20A model UV-visible detector, CTO-20AC model oven, CBM-20A model interface and automatic injector with an SIL-20A model autosampler. Separations were carried out with a Shim-pack VP-ODS-C18 column (250 mm x 4.6 mm) connected to a pre-column Shim-pack Column Holder (10 mm x 4.6 mm).

The mobile phase consisted of solutions of acetic acid in 2% water (A) and methanol:water:acetic acid (70: 28: 2, v:v:v) (B). The analyses were performed with total time of 65 minutes, temperature of 40 ºC, flow rate of 1.0 mL min^{-1}, wavelength of 280 nm and injection volume of 20 µL in gradient elution (starting at 100% of solvent A up to 5 minutes; 70% of solvent A from 5 to 25 minutes; 60% of solvent A from 25 to 43 minutes; 55% of solvent A from 43 to 50 minutes and 0% of solvent A for 10 minutes) (Marques et al. 2016). The standards used were: ferulic acid, salicylic acid, syringic acid, gallic acid, *p*-coumaric acid, epicatechin, catechin, epicatechin gallate, resveratrol and quercetin.

The lyophilized extracts, dissolved in water (1:16, w:v) and the standards were filtered through a membrane of 0.45 µm (Millipore®) and injected into the chromatograph. Phenolic compounds were identified by comparison with retention times with standards. Quantification was accomplished through the construction of analytical curves obtained by linear regression, considering the coefficient of determination (R^2) of 0.99.

SNAKE VENOMS

Crystallized bulk venoms of the species *Bothrops moojeni, Bothrops atrox*, *Crotalus durissus terrificus* and *Lachesis muta muta* were bought from the Bioagentes serpentarium (Batatais –SP).

All venoms were previously assessed at different doses for all tests to establish the minimum inducing doses for each activity, considering scientific content of the Toxinology field. Thus, for each assay, different venoms were established, considering the most effective in inducing the effects, to assess the inhibiting effect of extracts of JSF.

HUMAN BIOLOGICAL MATERIAL

The protocols for the trials in which human cells or blood components were used previously assessed and approved by the Ethics Committee on Human Research (COEP) at the Federal University of Lavras under the registration number CAAE: 64329117.9.0000.5148.

The human blood used for the tests of hemolytic and thrombolytic activity and coagulation was obtained from healthy volunteers who had not used any medication for a period of 30 days before the collection. The blood was collected by venipuncture in tubes containing heparin for hemolytic activity, citrate for coagulant and anticoagulant-free activity for thrombolytic activity.

PHOSPHOLIPASE AND HEMOLYTIC ACTIVITY

The phospholipase and hemolytic activities were evaluated in solid medium as described by Habermann and Harm (1972) and Gutiérrez et al. (1988), respectively. The gel used for the assessment of phospholipase activity was prepared with $CaCl_2$

0.01 mol L^{-1}; 1:3 (v:v) egg yolk lecithin; phosphate-buffered saline (PBS), pH 7.4; 1% bacteriological agar and 0.005% sodium azide, and the medium was poured at a temperature of 45-50 ºC in petri dishes. After the gel solidification, the treatments were applied to holes and the dishes were kept in a cell cultivation chamber for 12 hours at 37 ºC.

The phospholipases A_2 trials were carried out using venoms of *B. moojeni, B. atrox* and *C. durissus terrificus*, in which the minimum phospholipase dose (30 µg) of each venom was previously incubated with the aqueous and methanolic extracts of JSF for 30 minutes at 37 ºC at the proportions of 1:0.1; 1:0.5; 1:1; 1:2.5; 1:5; 1:10 and 1:20 (venom: extract, w:w).

For the hemolytic activity, the gel was developed by replacing the lecithin from egg yolk by a human red cell concentrate in the same volume. To obtain the cells, the newly collected blood was centrifuged at 700 x *g* for 5 minutes. The plasma was, then, removed and the red cells were suspended in 5 mmol L^{-1} PBS, pH 7.4, and centrifuged under the same conditions, being this washing step repeated twice. The inhibition of the hemolytic activity was evaluated for the venoms of *B. moojeni* and *C. durissus terrificus*, these being previously incubated at the minimum hemolytic dose (30 µg) with the aqueous and methanolic extracts of JSF for 30 minutes at 37 ºC at the proportions of 1:0.1; 1:0.5; 1:1; 1:2.5 and 1:5 (venom:extract, w:w). Controls containing only venoms or the extracts were carried out.

The diameter of translucent halo around the hole in the gel was measured and the results were converted in percentage (%). The controls containing only venoms were considered as 100% of activity.

THROMBOLYTIC ACTIVITY

The thrombolytic activity was evaluated upon human blood clots formed *in vitro* according to the methodology described by Cintra et al. (2012). The clots were incubated for 24 hours at 37 ºC with samples containing venoms of *B. moojeni* and *C. durissus terrificus*, PBS or venom (at the minimum thrombolytic dose of 40 µg) previously incubated (30 minutes at 37 ºC) with aqueous and methanolic extracts of JSF at the proportions at 1:0.5; 1:1; 1:2.5 and 1:5 (venom:extract, w:w). The activities were estimated by measuring the volume of liquid released by each clot, the controls containing only venoms were considered as having 100% activity, and the average of the volumes obtained in the negative control (PBS) was subtracted from the other treatments.

COAGULANT/ANTICOAGULANT ACTIVITY

Evaluation of the coagulation time was performed as described by Rodrigues et al. (2000). The aqueous and methanolic extracts of JSF were previously incubated with the venoms of *B. moojeni* and *L. muta muta* for a period of 10 minutes at 37 ºC at the proportions of 1:0.5; 1:1; 1:2.5 and 1:5 (venom:extract, w:w). Tubes containing citrated plasma (200 µL) were kept at a 37 ºC bath. Incubated samples were added to the plasma and time was recorded until the formation of the clot. Controls containing only the extracts were also carried out. The minimum coagulant dose was previously established, which was the smallest venom quantity capable of inducing coagulation in a period between 50 and 180 seconds (Selistre et al. 1990).

STATISTICAL ANALYSIS

The results were presented as the average of six runs ± standard deviation. The data of phospholipase, hemolytic and thrombolytic activity were statistically evaluated by analysis of variance, and the averages were compared using the Dunnett test ($p<0.05$) with help of the statistical software (R Core Team 2012).

RESULTS AND DISCUSSION

The JSF presented moisture content of 9.28 ± 0.19% and the extraction yields (%), in dry weight, of the aqueous and methanolic extractions were of 55.02 ± 1.02 and 67.03 ± 1.32, respectively. The highest yield, in dry weight, was observed for the methanolic extraction.

The results of phytochemical screening indicate the presence of different metabolic groups of food and pharmacological interest in the aqueous and methanolic JSF extracts, such as tannins, flavonoids and catechins. Cardiac glycosides or steroids and alkaloids were not detected in the extracts. However, in addition to the metabolites of interest, small amounts saponins were found in the methanolic extract. Saponins are metabolites with antioxidant activity, able to combating the action of free radicals in the cell, and it is known to present cytotoxic activity against tumor cells (Schenkel et al. 2007, Leung and Wong 2010). Further benefits of the intake of saponins are related to a reduction in the risk of developing cardiovascular diseases, and as an antiviral agent, acting by inhibiting viral replication (Trugo et al. 2003).

The phenolic compounds in the aqueous and methanolic JSF extracts are shown in Figures 1 and 2 and Table I. The methanolic extract presented contents of phenolic compounds higher than those obtained for the aqueous extract. The extracts also presented different constitutions. For example, syringic acid was not detected in the aqueous extract and quercetin was not detected in the methanolic extract.

In the past decade, phenolic compounds have gained great interest in the medical field. Many important pharmacological properties such as cytotoxic and chemoprotective effects were attributed to this class of compounds (Dai and Mumper 2010). Increasing evidence suggests that the long-term ingestion of these compounds may result in favorable effects on the incidence of some diseases, such as cancer, cardiovascular disease, type II diabetes, and nervous system diseases, which frequently occur in the population (Spencer and Crozier 2012, Zhang and Rong 2016, Lin et al. 2017).

Phenolic compounds of plants, fruits and agro-industrial residues have pharmacological properties that can be used for the design of natural therapeutic agents. Studies using extracts of grape

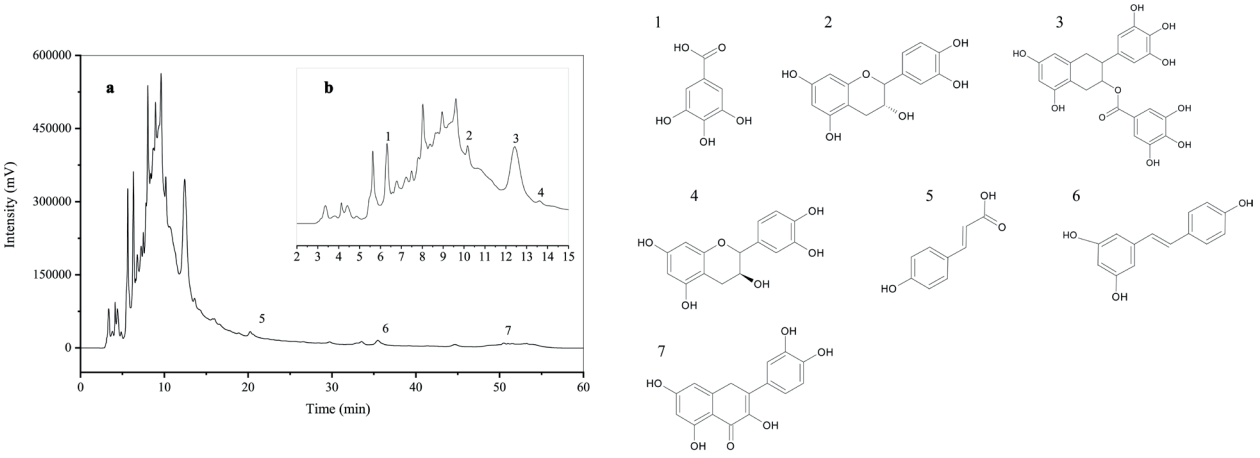

Figure 1 - (**a**) Chromatogram of jabuticaba skin flour aqueous extract, with peak identification: 1. gallic acid (time = 6.311 min); 2. catechin (time = 10.127 min); 3. epigallocatechin gallate (time = 12.418 min); 4. epicatechin (time = 13.602 min); 5. *p*-coumaric acid (time = 20.238 min); 6. resveratrol (time = 35.462 min) and 7. quercetin (time = 51.780 min). (**b**) Expansion of the chromatogram at times from 2 to 15 min.

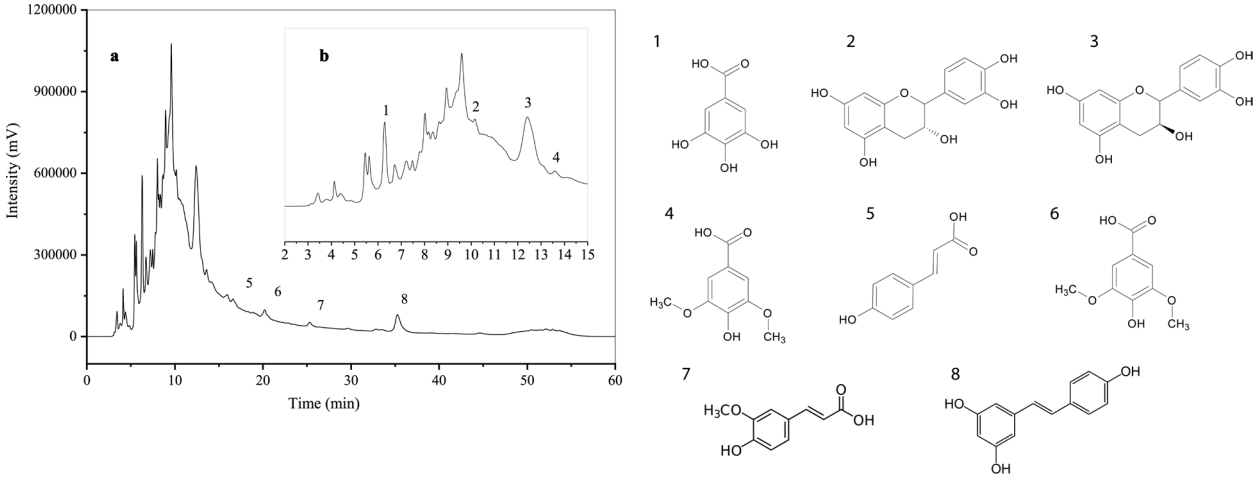

Figure 2 - (**a**) Chromatogram of jabuticaba skin flour aqueous extract, with peak identification: 1. gallic acid (time = 6.281 min); 2. catechin (time = 10.150 min); 3. epigallocatechin gallate (time = 12.405 min); 4. epicatechin (time = 13.587 min); 5. syringic acid (time = 15.936 min); 6. *p*-coumaric acid (time = 20.183 min); 7. ferulic acid (time = 25.299 min) and 8. resveratrol (time = 35.263 min). (**b**) Expansion of the chromatogram at times from 2 to 15 min.

TABLE I
Phenolic compounds content in aqueous and methanol extract of jabuticaba skin flour.

Phenolic compounds (mg L^{-1})	**Aqueous extract**	**Methanolic extract**
Gallic acid	65.18 ± 1.42	132.05 ± 1.29
Catechin	33.96 ± 0.19	277.62 ± 2.94
Epigallocatechin gallate	517.83 ± 1.69	724.73 ± 2.89
Epicatechin	10.45 ± 0.07	37.57 ± 0.49
Siringic acid	-	5.72 ± 0.09
p-Cumaric acid	1.44 ± 0.01	4.03 ± 0.04
Resveratrol	3.27 ± 0.02	25.97 ± 0.92
Quercetin	0.07 ± 0.00	-
∑ Phenolic compounds	632.20	1,207.69

The results correspond to the averages of the triplicates and the standard deviation.

seeds and skin identified bioactive compounds in both *in vitro* and *in vivo* activities. The authors identified flavonoids which protected platelets from peroxidative stress, modulated platelet function, and reduced the release of reactive oxygen intermediates, suggesting antithrombotic and anti-inflammatory properties. The *in vivo* results suggest the consumption of these compounds to prevent degenerative processes through their incorporation into functional foods, nutraceuticals, and cosmetics (Vitseva et al. 2005, Teixeira et al. 2014).

Flavonoids such as quercetin, catechin and epicatechin have anti-inflammatory and antithrombogenic properties. The main anti-inflammatory effects of flavonoids occur through the interaction with various enzymatic systems and the elimination of free radicals, in addition to the inhibition of arachidonic acid metabolism (Manach et al. 2005). As for antithrombogenic effects, flavonoids are inserted into the catalytic site of thrombin, blocking its activity (Mozzicafreddo et al. 2006). These phenolic compounds were identified in the extracts of JSF.

Studies using jabuticaba skin already report human health benefits due to its phenolic composition. The administration of jabuticaba skin flour in rats prevented lipid peroxidation and increased their antioxidant defense (Lage et al. 2014, Batista et al. 2014, Lenquiste et al. 2015). Already the administration of jabuticaba skin for 4 weeks in humans decreased serum levels of oxidative peroxidation (Batista et al. 2016). Alves et al. (2017) prepared restructured hams with the addition of JSF and the results showed an increase in fiber content and phenolic compounds, with good acceptance, provided that the fact it had antioxidants and dietary fibers was informed to the consumer. The authors related these benefits to the presence of phenolic compounds.

Figure 3 shows the phospholipase activity (%) induced by venoms of snakes from the *B. moojeni* (a), *B. atrox* (b), and *C. durissus terrificus* (c) species previously incubated with aqueous and methanolic JSF extract.

The aqueous and methanolic extracts, at the lowest ratios tested (1:0.1; 1:0.5; 1:1; 1:2.5), inhibited the phospholipase activity induced by *B. moojeni* venom, and the highest inhibition was observed at the 1:0.1 ratio of 26% and 22%, respectively (Fig. 3a).

The aqueous extract showed a significant inhibitory action on phospholipase activity induced by *B. atrox* venom from the ratio of 1:0.1, reducing the activity of this venom at 21%. The other proportions of aqueous extract also presented a significant inhibitory action on phospholipase activity, but were lower and did not differ among them. The same was observed for the methanolic extract. All proportions of methanolic extract presented a significant inhibitory action on phospholipase activity induced by *B. atrox* venom, but were lower (approximately 11%) and did not differ among them (Fig. 3b).

For the venom of *C. durissus terrificus*, inhibition was observed after incubation with the aqueous extract at the ratios 1:10 and 1:20, of 41% and 100%, respectively. The methanolic extract induced significant inhibitions at the ratios 1:1, 1:2.5, 1:5 and 1:10, with the highest inhibition observed at 1:20, reducing phospholipase activity by approximately 33% (Fig. 3c).

Phospholipases A_2 are considered important keys in the release of arachidonic acid, and are precursors of pro-inflammatory mediators, such as prostaglandins and leukotrienes. These enzymes are responsible for changes in hemostasis (Gutiérrez and Lomonte 2013), thus involved in a number of diseases such as asthma and allergic reactions (Pniewska et al. 2014), arthritis (Kokotos et al. 2014), atherosclerosis (Ait-Oufella et al. 2014), cancer (Berg et al. 2001), neurological diseases such as Alzheimer (Sagy-Bross et al. 2014), multiple sclerosis (Kalyvas and David 2004) and Parkinson (Lee et al. 2010).

Several plant extracts have been described with inhibitory activity on phospholipases A_2 and being efficient in inhibiting or preventing inflammatory processes, aiding in the management of inflammatory diseases and this inhibitory activity was attributed to phenolic compounds (Kishore et al. 2016, Silva et al. 2016, Moura et al. 2017, Kuganesan et al. 2017).

According to Moura et al. (2016), tannins can form complexes with calcium, which is a cofactor of phospholipases A_2 and various enzymes involved in the coagulation cascade. On the other hand, flavonoids, are able to inhibit the action of phospholipases A_2 inhibiting the cyclooxygenase and lipoxygenase pathways and the release of arachidonic acid, thereby decreasing the formation of inflammatory metabolites (Serafini et al. 2011, Nworu and Akah 2015). In the aqueous and methanolic JSF extracts evaluated in this study, phenolic compounds such as tannins, phenolic acids and flavonoids were identified, thus explaining the inhibition of phospholipase activity and, therefore, they may act to inhibit the release of inflammatory

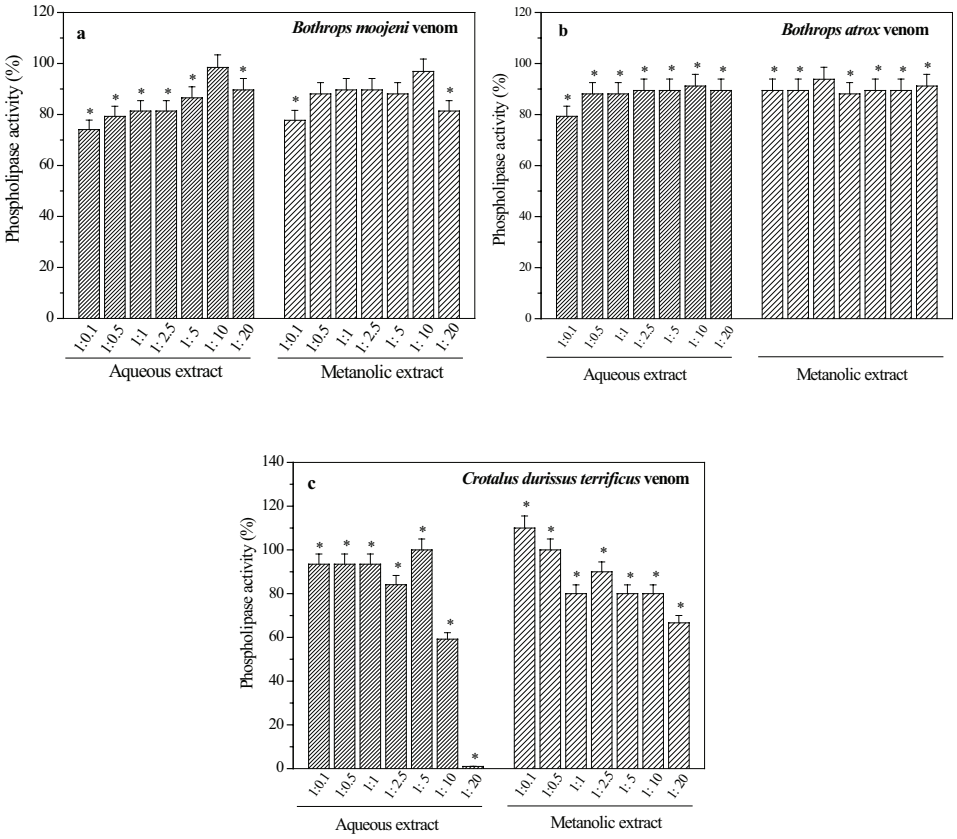

Figure 3 - Phospholipase activity (%) induced by the venoms of snakes from the species: *Bothrops moojeni* (**a**), *Bothrops atrox* (**b**) and *Crotalus durissus terrificus* (**c**), previously incubated with aqueous and methanolic extracts of jabuticaba skin. Controles (+) containing only venom (30 µg) was considered as having 100% of activity. The results correspond to the average of triplicates of the data obtained for each proportion (venom: extract, w:w) and the standard deviations. *the averages differ statistically from the respective positive control by the Dunnett test ($p<0.05$).

mediators. *In vivo* tests using these extracts are required to substantiate this action.

Figure 4 shows the hemolytic activity in solid medium. This assay was performed to evaluate the direct effects of extracts on erythrocyte membranes, as well as the potential of extracts to inhibit venom-induced cytotoxicity. It was observed that the extracts alone did not induce hemolysis, at the concentration and incubation time conditions evaluated.

The aqueous extract showed a significant inhibitory action on the hemolysis induced by *B. moojeni* venom from the ratio of 1:1, reducing the activity of this venom between 21% and 55%. The methanolic extract provided significant inhibition from the ratio 1:0.5, reducing the action of this venom from 41% to 53% (Fig. 4a). For the venom of *C. durissus terrificus*, incubation with the aqueous extract also showed significant inhibitory action, inhibiting 69% at the ratio 1:5. The methanolic extract reduced the activity of this venom by 21% to 100%, from the ratio 1:0.5 (w:w) (Fig. 4b). The lysis of erythrocytes was decreased with the increase in the proportion of extracts. The data show a greater inhibitory action of the methanolic extract, when incubated with the venoms of *B. moojeni* and *C. durissus terrificus* and, even with

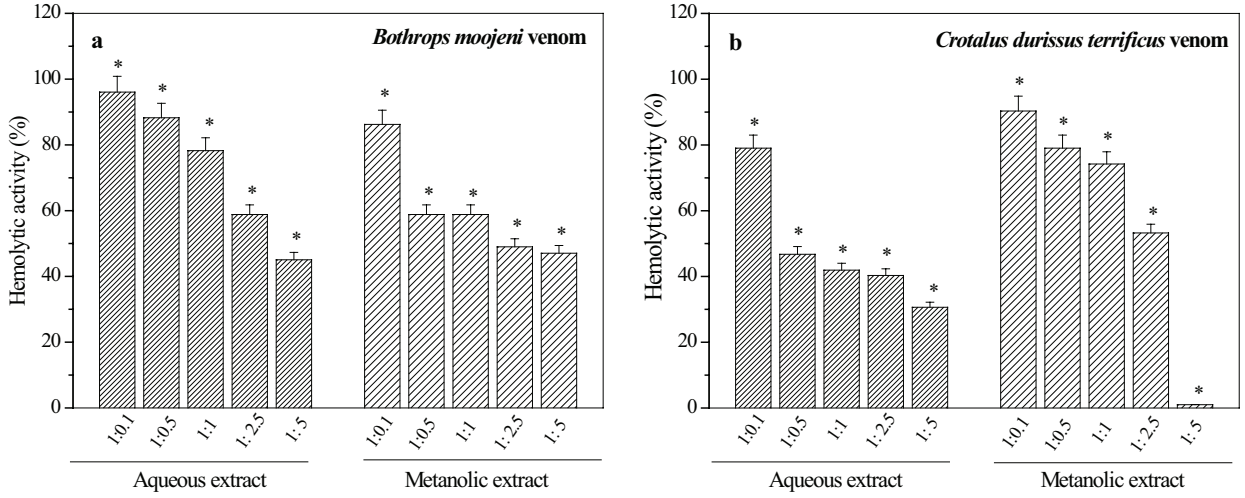

Figure 4 - Hemolytic activity (%) induced by the venoms of snakes from the species: *Bothrops moojeni* (**a**) and *Crotalus durissus terrificus* (**b**) previously incubated with aqueous and methanolic extracts of jabuticaba skin. Controles (+) containing only venom (30 µg) was considered as having 100% of activity. The results correspond to the average of triplicates of the data obtained for each proportion (venom: extract, w:w) and the standard deviations. *the averages differ statistically from the respective positive control by the Dunnett test ($p<0.05$).

the identification of saponins in this extract, its low content did not have able to cause the release of hemoglobin and induce hemolysis.

The enzymes involved in the hemolytic activity are serine proteases and metalloproteases that degrade membrane proteins and the phospholipases A_2 (responsible for the breakdown of phospholipids that make up the cell membranes). The action of these enzymes destabilizes the membrane structure, altering the ion flow and intracellular metabolism, thus resulting in the lysis of red blood cells (Garcia Denegri et al. 2010, Gutiérrez and Lomonte 2013). Therefore, the inhibition of hemolytic activity is also a proof of the action potential of the extracts evaluated on the inflammatory response and the blood coagulation cascade.

The inhibitions of the enzymes phospholipases A_2 and proteases presented in this work may be related on to fact that the phenolic compounds present in extracts have synergistic and additive effects. This could create variations in the mode of action depending on their concentration. The reflex of such variations is oscillations on the results obtained on phospholipase and hemolytic assays when increasing the concentrations of both extracts (Lidija 2015, Ozdal et al. 2013). Another consideration is related to the fact that phenolic compounds have able to promote conformational changes in enzymes, resulting in a diminishing or increasing in its activity based on the type of modification. That is, some compounds could bind to the active site, or a coordination site, leading to a decrease or inhibition of the activity. Conversely, other compounds could stabilize and enhance the catalytic activity of such enzymes.

Fig. 5 shows the thrombolytic activities of snake venoms of species *B. moojeni* and *C. durissus terrificus*, previously incubated with the aqueous and methanolic JSF extract.

The activity of *B. moojeni* venom decreased in approximately 49, 66, 79 and 83%, after incubation with the aqueous extract at ratios 1:0.5, 1:1, 1:2.5 and 1:5, respectively. Significant inhibitions were also observed after incubation of this venom with the methanolic extract, with reductions of 51, 59, 68 and 83% at the ratios of 1:0.5, 1:1, 1:2.5 and 1:5, respectively (Fig. 5a). Thus, the highest concentrations of extracts were responsible for the

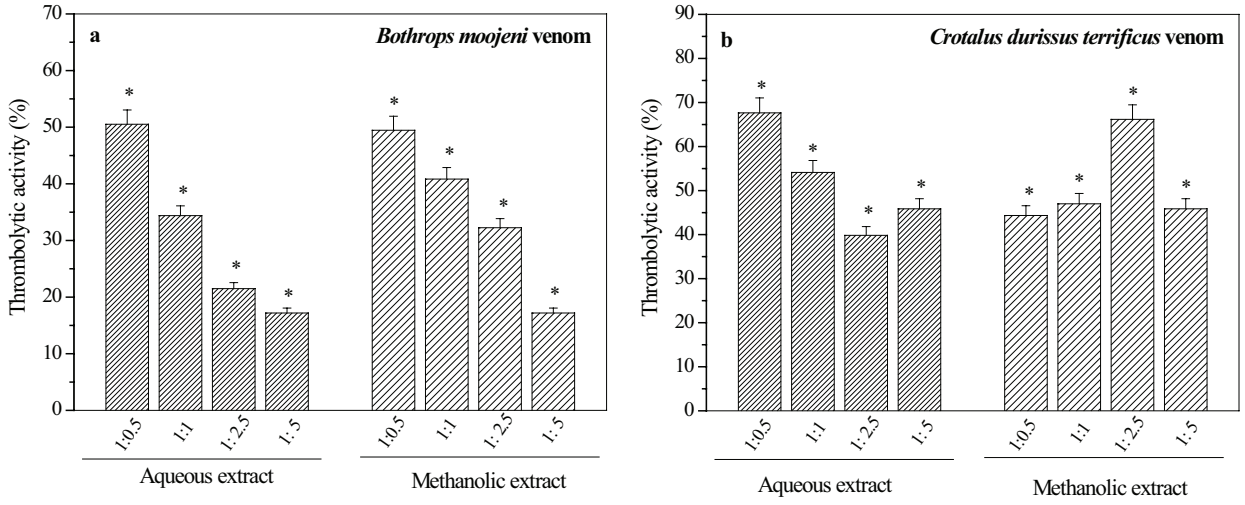

Figure 5 - Thrombolytic activity (%) induced by the venoms of snakes from the species: *Bothrops moojeni* (**a**) and *Crotalus durissus terrificus* (**b**) previously incubated with aqueous and methanolic extracts of jabuticaba skin. Controles (+) containing only venom (40 µg) was considered as having 100% of activity. The results correspond to the average of triplicates of the data obtained for each proportion (venom: extract, w:w) and the standard deviations. *the averages differ statistically from the respective positive control by the Dunnett test ($p<0.05$).

most significant inhibitions, possibly due to the concomitant increase in the concentration of the phenolic compounds present in the extract.

For *C. durissus terrificus* venom, thrombolytic activity was inhibited in 32, 46, 60 and 54%, after incubation with the aqueous extract at ratios 1:0.5, 1:1, 1:2.5 and 1:5, respectively, indicating that the increase in the extract to the ratio of 1:2.5 resulted in a higher inhibition (Fig. 5a). Significant inhibitions were also observed after incubation with the methanolic extract, showing that the 1:2.5 ratio had the lowest inhibition of thrombolytic activity, with 34% (Fig. 5b).

Thrombosis occurs in abnormal hemostatic conditions due to the uncontrolled action of thrombin on fibrinogen, or excessive production of thrombin (Majumdar et al. 2014). These disorders, related to the imbalance between fibrin formation and fibrinolysis, are the main responsible for the high rate of morbidity and mortality. Platelet aggregation contributes to the development of atherosclerosis and acute platelet thrombus formation, followed by artery embolization. In the case of inflammation, arachidonic acid is released and metabolized by platelets to form prostaglandin, endoperoxides and thromboxane A_2, leading to platelet activation and aggregation (Gadek-Michalska et al. 2013, Sivamani 2014). Thus, it is necessary to evaluate natural extracts or isolated active principles capable of inhibiting enzymes that act in the blood clotting cascade, such as thrombin, as well as in platelet aggregation, such as phospholipases A_2 and proteases.

Elumalai et al. (2012) described the induction of thrombolytic activity by the methanolic extract of *Bougainvillea glabra* and mentioned that metabolite groups in the extracts, such as tannins, flavonoids, saponins, glycosides and terpenes, may be related to thrombus dissolution, due to the combined effect of active compounds, being able to perform the activity by different mechanisms. According to the phytochemical screening, tannins, flavonoids, saponins and terpenes were identified in JSF extracts.

The induced coagulation of citrated plasma was performed using pre-determined minimum coagulant doses of 5µg and 10 µg of *B. moojeni* and *L. muta muta* venoms, respectively. All values

that differed from the positive control in time equal to or greater than 10 seconds were considered significantly different, since this time is enough to trigger some processes of the coagulation cascade, such as prothrombin activation (normally between 10 and 14 seconds) and the partial activation of thromboplastin (normally between 24 and 40 seconds), according to a coagulogram.

The aqueous and methanolic JSF extracts previously incubated with *B. moojeni* venom at ratios 1:1, 1:2.5, 1:5 and 1:10, showed anticoagulant action, inducing increases in coagulation time from 12 to 91 seconds (Table II).

As for *L. muta muta* venom, incubations with aqueous and methanolic extracts resulted in an increase in coagulation time, characterizing anticoagulant action for all evaluated ratios (1:0.5, 1:1, 1:2.5, 1:5 and 1:10), with periods of 15 to 67 seconds and 35 to 59 seconds higher than the control, respectively (Table II).

The prolongation of coagulation time (Table II) and dissolution of blood clots (Fig. 5), mainly related to coagulant enzymes inhibition, may also be due to the action of flavonoids present in JSF extracts. Flavonoids act on the production of nitric oxide in blood platelets, thus inhibiting platelet aggregation and retarding clot formation (Silva et al. 2011).

Blood coagulation is a physiological process to prevent blood loss caused by vascular injury. This process leads to the generation of thrombin, a serine protease that plays a central role in the control of hemostasis and is responsible for the conversion of fibrinogen to fibrin, platelet activation and other coagulation factors (Crawley et al. 2007, Licari and Kovacic 2009). Thrombin is one of the most important enzymes in hemostasis, since it acts in the regulation of plasma coagulant activity. Disorders in mechanisms controlling the generation and activity of thrombin contribute to the formation and development of many cardiovascular diseases (atherosclerosis, coronary heart disease, venous thromboembolism, and stroke) (Bijak et al. 2011).

The enzymes present in venoms, such as some hemorrhagic metalloproteinases, act as anticoagulants; others, fibrinogenolytic, act as procoagulants, generating fibrin networks; serine proteases responsible for the formation of friable

TABLE II
Effect of extracts of jabuticaba skin flour on the coagulant activity induced by different snake venoms.

	Proportion venom: extract (m:m)	Clotting time (s)	
		Bothrops moojeni	***Lachesis muta muta***
Aqueous extract	1:0.5	65.00 ± 3.79	107.33 ± 2.31[a]
	1:1	85.00 ± 2.65[a]	107.33 ± 3.79[a]
	1:2.5	116.00 ± 1.15[a]	124.00 ± 2.65[a]
	1:5	129.00 ± 2.89[a]	135.67 ± 3.21[a]
	1:10	128.00 ± 2.31[a]	159.67 ± 3.79[a]
Methanolic extract	1:0.5	81.00 ± 2.52	97.30 ± 2.08
	1:1	131.00 ± 3.46[a]	127.67 ± 1.15[a]
	1:2.5	135.00 ± 2.89[a]	142.67 ± 1.53[a]
	1:5	164.00 ± 3.00[a]	151.30 ± 2.89[a]
	1:10	162.00 ± 1.53[a]	147.00 ± 2.00[a]
Control		**73.00 ± 2.65***	**92.30 ± 2.08****

*The control was carried out with 5 µg each evaluated venom. **The control was carried out with 10 µg each evaluated venom. The results are presented as the average of triplicates ± standard deviation. [a] differs from the respective positive controls in values equal to or above 10 seconds.

clots can also act as anticoagulants, but with less efficiency, and still some phospholipases A_2 can act as procoagulants. In addition, the use of phospholipases A_2 may act as a procoagulant (Cate et al. 2017). Thus, finding plant extracts that show inhibitory activity of phospholipases A_2, metalloproteases and serine proteases is of great relevance in the pathophysiological context of maintenance and/or disorders in hemostasis. The aqueous and methanolic JSF extracts were able to increase coagulation time by inhibiting coagulant enzymes, when previously incubated with different venoms, and could be used as anticoagulant agents of wide application in the treatment of cardiovascular diseases. However, further studies are necessary to comprehend such actions allowing the evaluation of their effective and safe doses and formulations of use; and thus, affirm the nutraceutical potential exerted by this jabuticaba residue.

CONCLUSION

The aqueous and methanolic extracts of jabuticaba skin flour were capable of modulating the enzymatic activity of snake venoms by inhibiting or potentiating phospholipases A_2 and proteases (mainly thrombin-like). This occurs probably due to the presence of phenolic compounds able interact with catalytic sites of the enzymes, or a coordination site, leading to a decrease or inhibition of these enzymes activity.

In this way, the extracts of jabuticaba skin flour stand out for its anti-genotoxic potential and modulator of processes related to hemostasis. However, new studies are still needed to expand the knowledge regarding the mechanisms of interactions between the bioactive compounds and enzymes, cellular components or other animal molecules.

ACKNOWLEDGMENTS

The authors would like to thank Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the grants provided.

AUTHOR CONTRIBUTIONS

T.R. Marques contributed in running the laboratory work, analysis of the data and drafted the paper. M.A. Braga contributed in running the laboratory work and to critical reading of the manuscript. P. H.S. Cesar contributed in running the laboratory work. S. Marcussi supervised the laboratory work and contributed to critical reading of the manuscript. A.D. Corrêa designed the study and supervised the laboratory work and contributed to critical reading of the manuscript.

REFERENCES

ABE LT, LAJOLO FM AND GENOVESE MI. 2012. Potential dietary sources of ellagic acid and other antioxidants among fruits consumed in Brazil: jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg). J Sci Food Agric 92: 1679-1687.

AIT-OUFELLA H, MALLAT Z AND TEDGUI A. 2014. Lp-PLA_2 et sPLA_2 Biomarqueurs cardiovasculaires. Med Sciences 30: 526-531.

ALVES APC, CORREA AD, OLIVEIRA FC, ISQUIERDO PE, ABREU CMP AND BORÉM FM. 2014. Influence of drying temperature on the chemical constituents of jaboticaba (*Plinia Jaboticaba* (Vell.) Berg) skin. Acta Sci Technol 36: 721-726.

ALVES APC, CORREA AD, PINHEIRO ACM AND OLIVEIRA FC. 2013. Flour and anthocyanin extracts of jaboticaba skins used as a natural dye in yogurt. IJFST 48: 2007-2013.

ALVES APC, MARQUES TR, CARVALHO TCL, PINHEIRO ACM, RAMOS EM AND CORREA AD. 2017. Elaboration and acceptability of restructured hams added with jabuticaba skin. Food Sci Technol 37: 232-238.

AOAC - ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 2016. Official methods of analysis, 19 ed., Gaithersburg, 3000 p.

ASCHERI DPR, ASCHERI JLR AND CARVALHO CWP. 2006. Caracterização da farinha de bagaço de jabuticaba e propriedades funcionais dos extrusados. Food Sci Technol 26: 897-905.

BABBAR N, OBEROI HS AND SANDHU SK. 2015. Therapeutic and nutraceutical potential of bioactive

compounds extracted from fruit residues. J Crit Rev Food Sci Nutr 55: 319-337.

BATISTA ÂG ET AL. 2014. Intake of jaboticaba peel attenuates oxidative stress in tissues and reduces circulating saturated lipids of rats with high-fat diet-induced obesity. J Funct Foods 6: 450-461.

BATISTA ÂG, ZANZER YC, MARÓSTICA JÚNIOR MR AND OSTMAN EM. 2016. Four-weeks intervention with jaboticaba peel lowers MDA levels after post-prandial challenge in healthy adults. Proc Nut Soc 75: E78.

BERG OG, GELB MH, TSAI MD AND JAIN MK. 2001. Interfacial enzymology: the secreted phospholipase A_2-Paradigm. Chem Rev 101: 2613-2654.

BIJAK M, BOBROWSKI M, BOROWIECKA M, PODSEDEK A, GOLANSKI J AND NOWAK P. 2011. Anticoagulant effect of polyphenols-rich extracts from black chokeberry and grape seeds. Fitoterapia 82: 811-817.

BRAUD SB AND WISNER AC. 2000. Snake venom proteins acting on hemostasis. Biochimie 82: 851-859.

CATE HT, HACKENG TM AND FRUTOS PG. 2017. Coagulation factor and protease pathways in thrombosis and cardiovascular disease. J Thromb Haemost 117: 1265-1271.

CECHETTI S, SPADARO F, GESSANI S, PODO F AND FANTUZZI L. 2017. Phospholipases: at the crossroads of the immune system and the pathogenesis of HIV-1 infection. JLB 101: 53-75.

CINTRA ACO, DE TONI LGB, SARTIM MA, FRANCO JJ, CAETANO RC, MURAKAMI MT AND SAMPAIO SV. 2012. Batroxase, a new metalloproteinase from *Bothrops atrox* snake venom with strong fibrinolytic activity. Toxicon 60: 70-82.

CITADIN I, DANNER MA AND SASSO SAZ. 2010. Jabuticabeiras. Rev Bras Frutic 32: 343-656.

CRAWLEY JT, ZANARDELLI S, CHION CK AND LANE DA. 2007. The central role of thrombin in hemostasis. J Thromb Haemost 5(Suppl 1): 95-101.

DAI J AND MUMPER R. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15: 7313-7352.

ELUMALAI A, CHINNA ESWARIAH M, VIDHYULATHA CHOWDARY CH, RANJITH K, ANUSHA M AND NARESH K. 2012. Screening of thrombolytic activity os Bougainvillea glabra leaves extracts by *in-vitro*. Asian J Res Pharm Sci 2: 134-136.

EVANS HM, HOWE PRC AND WONG RHX. 2016. Clinical evaluation of effects of chronic resveratrol supplementation on cerebrovascular function, cognition, mood, physical function and general well-being in postmenopausal women-rationale and study design. Nutrients 8: 150.

FORTES GAC, NAVES SS, GODOI FFF, DUARTE AR, FERRI PH AND SANTOS SC. 2011. Assessment of a maturity index in jabuticaba fruit by the evaluation of phenolic compounds, essential oil components, sugar content and total acidity. American J Food Technol 6: 974-984.

GĄDEK-MICHALSKA A, TADEUSZ J, RACHWALSKA P AND BUGAJSKI J. 2013. Cytokines, prostaglandins and nitric oxide in the regulation of stress-response systems. Pharmacol Rep 65: 1655-1662.

GARCIA DENEGRI ME, ACOSTA OC, HUANCAHUIRE-VEGA S, MARTINS-DE-SOUZA D, MARANGONI S, MARUÑAK SL, TEIBLER GP, LEIVA LC AND PONCE-SOTO LA. 2010. Isolation and functional characterization of a new acidic PLA_2 Ba SpII RP4 of the Bothrops alternatus snake venom from Argentina. Toxicon 56: 64-74.

GUTIÉRREZ JM, AVILA C, ROJAS E AND CERDAS L. 1988. An alternative *in vitro* method for testing the potency of the polyvalent antivenom produced in Costa Rica. Toxicon 26: 411-413.

GUTIÉRREZ JM AND LOMONTE B. 2013. Phospholipases A_2: unveiling the secrets of a functionally versatile group of snake venom toxins. Toxicon 62: 27-39.

HABERMANN E AND HARM KL. 1972. A sensitive and specific plate test for the quantitation of phospholipases. Anal Biochem 50: 163-173.

KALYVAS A AND DAVID S. 2004. Cytosolic phospholipase A_2 plays a key role in the pathogenesis of multiple sclerosis-like disease. Neuron 41: 323-335.

KISHORE V, YARLA NS, ZAMEER F, NAGENDRA PRASAD MN, SANTOSH MS, MORE SS, RAO DG AND DHANANJAYA BL. 2016. Inhibition of group IIA secretory phospholipase A_2 and its inflammatory reactions in mice by ethanolic extract of *Andrographis paniculata*, a well-known medicinal food. Pharmacognosy Res 8: 213-216.

KOKOTOS G, FEUERHERM AJ, BARBAYIANNI E, SHAH I, SÆTHER M, MAGRIOTI V, NGUYEN T, CONSTANTINOU-KOKOTOU V, DENNIS EA AND JOHANSEN B. 2014. Inhibition of group IVA cytosolic phospholipase A_2 by thiazolyl ketones *in vitro*, *ex vivo*, and *in vivo*. J Med Chem 57: 7523-7535.

KUGANESAN G, THIRIPURANATHAR AN, NAVARATNE AND PARANAGAMA PA. 2017. Antioxidant and anti-inflammatory activities of peels, pulps and seed kernels of three common mango (*Mangifera indical* L.) varieties in sri lanka. IJPSR 8: 70-78.

KUMAR K. 2015. Role of edible mushrooms as functional foods - a review. South Asian J Food Technol Environment 1: 211-218.

KUMAR K AND KUMAR S. 2015. Role of nutraceuticals in health and disease prevention: a review. South Asian J Food Technol Environment 1: 116-121.

LAGE FF, SIMÃO AA, GUEDES MNS, RAMOS VO, SOUSA RV AND DORRÊA AD. 2014. Jabuticaba [Pliniajaboticaba (Vell.) Berg] skins decrease lipid peroxidation: Hepatoprotective and antihyperlipidemic effects. African J Biotechnol 13: 1295-1302.

LEE HJ, BAZINET RP, RAPOPORT SI AND BHATTACHARJEE AK. 2010. Brain arachidonic

acid cascade enzymes are upregulated in a rat model of unilateral Parkinson disease. Neurochem Res 35: 613-619.

LENQUISTE SA, MARINELI RS, MORAES EA, DIONISIO AP, BRITO ES AND MARÓSTICA JÚNIOR MR. 2015. Jaboticaba peel and jaboticaba peel aqueous extract shows *in vitro* and *in vivo* antioxidant properties in obesity model. Food Res Int 77: 162-170.

LEUNG KW AND WONG AS. 2010. Pharmacology of ginsenosides: a literature review. American J Chin Med 5: 20.

LICARI LG AND KOVACIC JP. 2009. Thrombin physiology and pathophysiology. J Vet Emerg Crit Care (San Antonio) 19: 11-22.

LIDIJA J. 2015. Interactions of polyphenols with carbohydrates, lipids and proteins. Food Chem 15: 556-567.

LIMA AJB, CORRÊA AD, ALVES APC, ABREU CMP AND DANTAS-BARROS AM. 2008. Caracterização química da fruta jabuticaba (*M. cauliflora* Berg) e de suas frações. Arch Latinoam Nutr 58: 416-421.

LIN BW, GONG CC, SONG HF AND CUI YY. 2017. Effects of anthocyanins on the prevention and treatment of cancer. Br J Pharmacol 174: 1226-1243.

MAJUMDAR S, SARMAH B, GOGOI D, BANERJEE S, CHATTOPADHYAY P AND MUKHERJEE AK. 2014. Characterization, mechanism of anticoagulant action and asessment of therapeutic potential of a fibrinolytic serine protease (Brevithrombolase) purified from *Brevibacillus brevis* strain FF02B. Biochimie 103: 50-60.

MANACH C, WILLIAMSON G, MORAND C, SCALBERT A AND RÉMÉSY C. 2005. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr 81(1 Suppl): 230S-242S.

MARQUES TR, CAETANO AA, SIMÃO AA, CASTRO FCO, RAMOS VO AND CORRÊA AD. 2016. Metanolic extract of *Malpighia emarginata* bagasse: phenolic compounds and inhibitory potential on digestive enzymes. Rev Bras Farmacogn 26: 191-196.

MASUDA S, MURAKAMI M, KOMIYAMA K, ISHIHARA M, ISHIKAWA Y, ISHII T AND KUDO I. 2005. Various secretory phospholipase A_2 enzymes are expressed in rheumatoid arthritis and augment prostaglandin production in cultured synovial cells. FEBS Journal 272: 655-672.

MATOS FJA. 1997. Introdução à fitoquímica experimental. Fortaleza: UFC,141 p.

MOURA VM, SILVA WC, RAPOSO JD, FREITAS-DE-SOUSA LA, DOS-SANTOS MC, OLIVEIRA RB AND VERAS MOURÃO RH. 2016. The inhibitory potential of the condensed-tannin-rich fraction of *Plathymenia reticulata* Benth. (Fabaceae) against *Bothrops atrox* envenomation. J Ethnopharmacology 13: 136-142.

MOURA VM, SOUZA LYA, GUIMARÃES NC, SANTOS IGC, ALMEIDA PDO AND OLIVEIRA RB. 2017. The potential of aqueous extracts of *Bellucia dichotoma* Cogn. (Melastomataceae) to inhibit the biological activities of *Bothrops atrox* venom: A comparison of specimens collected in the states of Pará and Amazonas, Brazil. J Ethnopharmacology 196: 168-177.

MOZZICAFREDDO M, CUCCIOLONI M, ELEUTERI AM, FIORETTI E AND ANGELETTI M. 2006. Flavonoids inhibit the amidolytic activity of human thrombin. Biochimie 88: 1297-1306.

NANJARAJ UAN, YARISWAMY M, JOSHI V, NATARAJU A, GOWDA TV AND VISHWANATH BS. 2013. Implications of phytochemicals in snakebite management: presente status and future prospective. Toxin Reviews 33: 1-24.

NWORU CS AND AKAH PA. 2015. Anti-inflammatory medicinal plants and the molecular mechanisms underlying their activities. Afr J Tradit Complement Altern Med 12: 52-61.

OLAIYA CO, SOETAN KO AND ESAN AM. 2016. The role of nutraceuticals, functional foods and value added food products in the prevention and treatment of chronic diseases. African J Food Sci 10: 185-193.

OZDAL T, CAPANOGLU E AND ALTAY FILIZ. 2013. A review on protein–phenolic interactions and associated changes. Food Res Int 51: 954-970.

PELUSO AND SERAFINI M. 2017. Antioxidants from black and green tea: from dietary modulation of oxidative stress to pharmacological mechanisms. British J Pharmacology, 174(11): 1195-1208.

PNIEWSKA E, SOKOLOWSKA M, KUPRYS-LIPINSKA I, PRZYBEK M, KUNA P AND PAWLICZAK R. 2014. The step further to understand the role of cytosolic phospholipase A_2 alpha and group X secretory phospholipase A_2 in allergic inflammation: pilot study. BioMed Res Int 2014: 670814.

POZZI N, CHEN Z AND DI CERA E. 2016. How the linker connecting the two kringles influences activation and conformational plasticity of prothrombin. J Biol Chem 291: 6071-6082.

PRAKASH D, GUPTA C AND SHARMA G. 2012. Importance of Phytochemicals in nutraceuticals. J Med Res Development 1: 70-78.

R CORE TEAM. 2012. R: A Language and Environment for Statistical Computing. Viena: Viena: R Foundation for Statistical Computing.

RAMANADHAM S, HSU FF, ZHANG S, JIN C, BOHRER A, SONG H, BAO S, MA Z AND TURK J. 2004. Apoptosis of insulin-secreting cells induced by endoplasmic reticulum stress is amplified by overexpression of group VIA calcium-independent phospholipase A_2 ($iPLA_2$ beta) and suppressed by inhibition of $iPLA_2$ beta. Biochemistry 3: 918-930.

RODRIGUES VM, SOARES AM, GUERRA-SÁ R, RODRIGUES V, FONTES MR AND GIGLIO JR. 2000. Structural and functional characterization of neuwiedase, a nonhemorrhagic fibrin (ogen)olytic metalloprotease from *Bothrops neuwiedi* snake venom. Arch Biochem Biophys 381: 213-224.

SAGY-BROSS C, KASIANOV K, SOLOMONOV Y, BRAIMAN A, FRIEDMAN A, HADAD NA AND LEVY R. 2014. The role of cytosolic phospholipase $A_2\alpha$ in amyloid precursor protein induction by amyloid $beta_{1-42}$: implication for neurodegeneration. J Neurochem 132: 559-571.

SAJEVIC T, LEONARDI A AND KRIŽAJ I. 2011. Haemostatically active proteins in snake venoms. Toxicon 57: 627-645.

SANTHOSH MS, HEMSHEKHAR M, SUNITHA K, THUSHARA RM, JNANESHWARI S, KEMPARAJU K AND GIRISH KS. 2013. Snake venom induced local toxicities, plant secondary metabolites as an auxiliary therapy. Mini Rev Med Chem 13: 106-123.

SATO ACK AND CUNHA RL. 2009. Effect of particle size on rheological properties of jaboticaba pulp. J Food Engineering 91: 566-570.

SCHENKEL EP, GOSMAN G AND ATHAYDE ML. 2007. Saponinas. In: Simões CMO, Schenkel EP, Gosman G, Mello JCP, Mentz LA and Petrovick PR. Farmacognosia: Da Planta ao Medicamento. 6. Ed. Porto Alegre: Editora da UFRGS, 1104 p.

SELISTRE HS, QUEIROZ LS, CUNHA OAB, DE SOUZA GEP AND GIGLIO JR. 1990. Isolation and characterization of hemorrhagic, myonecrotic and edema-inducing toxins from *Bothrops insularis* (jararaca ilhoa) snake venom. Toxicon 28: 261-273.

SERAFINI M, DEL RIO D, YAO D N'D, BETTUZZI S AND PELUSO I. 2011. HEATH BENEFITS OF TEA. In: Benzie IFF and Wachtel-Galor S (Eds), Herbal Medicine: biomolecular and clinical aspects. 2nd edition. Boca raton (FL): CRC Press/Taylor & Francis.

SILVA MC, SOUSA E, DUARTE B, MARQUES F, CARVALHO F, RIBEIRO LMC AND PINTO MMM. 2011. Flavonoids with an oligopolysulfated moiety: a new class of anticoagulant agents. J Med Chem 54: 95-106.

SILVA TP, MOURA VM, SOUZA MCS, SANTOS VNC, SILVA KAMM AND MENDES MGG. 2016. *Connarus favosus* Planch.: An inhibitor of the hemorrhagic activity of *Bothrops atrox* venom and a potential antioxidant and antibacterial agent. J Ethnopharmacology 183: 166-175.

SIVAMANI RK. 2014. Eicosanoids and Keratinocytes in Wound Healing. Adv Wound Care 3: 476-481.

SPENCER JPE AND CROZIER A (Eds). 2012. In: Flavonoids and Related Compounds: Bioavailability and Function. Oxidative Stress and Disease, Vol. 30, edited by Packer L and Cadenas H. Boca Raton, FL: CRC Press.

TEIXEIRA A, BAENAS N, DOMINGUEZ-PERLES R, BARROS A, ROSA E, MORENO DA AND GARCIA-VIGUERA C. 2014. Natural bioactive compounds from winery by-products as health promoters: a review. Int J Mol Sci 15: 15638-15678.

TRUGO LC, BEAR D AND BEAR E. 2003. Lupin. In: Caballero B, Trugo LC and Finglas PM. Encyclopedia of food sciences and nutrition. 2nd ed., Academic Press, p. 3623-3629.

TRUSEVYCH EH AND MACNAUGHTON WK. 2015. Proteases and their receptors as mediators of inflammation-associated colon cancer. Curr Pharm Des 21: 2983-2992.

VITSEVA O, VARGHESE S, CHAKRABARTI S, FOLTS JD AND FREEDMAN JE. 2005. Grape seed and skin extracts inhibit platelet function and release of reactive oxygen intermediates. J Cardiovasc Pharmacol 46: 445-451.

WONG RHX AND EVANS HM. 2017. Menopause: Resveratrol supplementation reduces pain experience by postmenopausal women. BioMed Sci 24: 916-922.

ZHANG H AND RONG T. 2016. Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. Current Opinion Food Sci 8: 33-42.