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THE ROLE OF ENZYMES IN THE ANGIOGENIC ACTIVITY OF *Hancornia speciosa* LATEX

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Abstract

The *Hancornia speciosa* latex has shown angiogenic activity. Angiogenesis plays a major role in wound healing, and materials that stimulate this process could be used to develop drugs. This study aimed to explain the role of proteins in the *H. speciosa* serum fraction latex in angiogenesis. Hence, this material was treated with proteinase K and the proteins were inactivated. After protein inactivation, angiogenic activity was assessed with the chick chorioallantoic membrane assay. The result showed that the proteins in the serum fraction are responsible for angiogenic activity. Then, the total protein content in the serum fraction and its enzymatic activity were investigated. The low protein content observed in the *H. speciosa* serum fraction latex suggests that this biomaterial could be used to develop new drugs with a hypoallergenic response. Despite the low protein content, there was a significant enzymatic activity of at least three enzymes in the serum fraction latex: β-1,3 glucanase, β-glucosidase, and proteases. These enzymes seem to influence the healing process, assisting debridement, extracellular matrix remodeling, and collagen deposition, and decreasing the chances of contamination by microorganisms. In conclusion, the enzymes in the *H. speciosa* serum latex are associated with the angiogenic activity of this biomaterial and may be used to assist the wound healing process.

Keywords: Chick chorioallantoic membrane. Enzymes. Proteinase K. Wound healing.

1. Introduction

Angiogenesis is the growth of blood vessels from the existing vasculature (Adair and Montani 2010). It is a vital process for human growth and development, wound healing, and granulation tissue formation (Folkman 2003). For decades, researchers have been looking for products or strategies to stimulate angiogenesis and consequently accelerate the wound healing process (Singer and Clark 1999; Scheneider et al. 2019). Plants produce a variety of angiogenic compounds that may be important to develop wound-healing drugs (Karim et al. 2014; Wittenauer et al. 2015; Danciu et al. 2015). Among them, latex has shown high angiogenic potential (de Almeida et al. 2015). Latex is an organic milky fluid that flows out of some plants after a tissue injury (Konno 2011). The general functions of this exudate in plants are associated with

the excretion of waste metabolites, coverage of damaged tissue, and defense against pathogens (Konno 2011). Among latex with angiogenic activities is the Hancornia speciosa Gomes latex.

H. speciosa, popularly called mangabeira, is a native species from Brazil that belongs to the Apocynaceae family. It is typically found in the Amazon Rainforest, Caatinga, and Cerrado vegetation. Ethnobotanical studies have shown the use of the *H. speciosa* latex in traditional medicine for treating skin diseases, tuberculosis, fungal diseases, gastric ulcers, and bone fractures (Pott and Pott 1994; Neto and Guarim-Morais 2003; Macedo and Ferreira 2004; Sampaio and Nogueira 2006). Besides the use in traditional medicine, the literature shows the high angiogenic (Almeida et al. 2014; D'Abadia et al. 2020; Bonete et al. 2020), osteogenic (Floriano et al. 2016; dos Santos Neves et al. 2016), antioxidant (D'Abadia et al. 2020), and anti-inflammatory (Marinho et al. 2011) potential of the *H. speciosa* latex. Regarding the toxic potential, the *H. speciosa* latex does not show cytogenotoxic effects against human cells (Costa et al. 2023), animals (Almeida et al. 2014), and plants (Ribeiro et al. 2016).

For bioprospecting studies, it is important to identify which substances in latex present angiogenic activity. A previous study determined that the compounds responsible for the angiogenic activity are present in the *H. speciosa* serum (SE) fraction latex. This fraction contains various proteins, secondary metabolites, and lutein compounds. Among the different components in the serum fraction, this study aims to evaluate the influence of proteins on angiogenic activity. To test this hypothesis, the proteins in the serum were inactivated with the proteinase K enzyme, and angiogenic activity was evaluated with the chick chorioallantoic membrane (CAM) assay. This is one of the most used methods to study angiogenesis and its main advantages are being fast, low-cost, simple, and easy to observe; having good reproducibility; and not presenting major ethical concerns (Nowak – Sliwinska et al. 2014; Aleksandrowicz and Herr 2015; do Prado et al. 2019). After the CAM experiments confirmed the role of proteins in angiogenesis, biochemical analyses were performed to quantify the total protein and the main enzyme classes in the H. speciosa serum fraction latex. The enzymatic activity of Hevea brasiliensis serum fraction latex was also quantified, and the results were compared with those of H. speciosa. The H. brasiliensis latex was selected as the control because it has proven angiogenic and wound-healing activities (Penhavel et al. 2016). The enzymatic profile of the H. speciosa biomaterial may provide a clue to the biological activity of the serum fraction in angiogenesis and consequent wound healing.

2. Material and Methods

Latex collection and extraction

The *H. speciosa* latex was obtained from trees of the collection of the State University of Goiás, in the city of Ipameri, state of Goiás, Brazil (17°43’19’’S, 48°9’35’’W, 773 m). Botanical identification was performed and a voucher specimen was deposited at the herbarium of the State University of Goiás (Anápolis, Goiás, Brazil) under number 4875. The latex was extracted in a sterile tube by drilling the tree trunk, according to the methodology by Arruda et al. (2016).

Latex fractionation

The H. speciosa latex was centrifuged at 4°C for 1 hour at 22,000 g (Heraeus Megafuge 16R, Thermo Scientific). After the initial centrifugation, the rubber was carefully separated and centrifuged again. Two different fractions were obtained after the centrifugations: an upper white area with rubber particles and an aqueous fraction *called serum (SE) fraction.*

The Hevea brasiliensis latex was used as a reference sample in the total protein quantification and enzymatic activity experiments. Thus, the same fractionation method was used for the *Hevea brasiliensis* latex to obtain the serum fraction.

Protein inactivation with proteinase K

Proteinase K, also known as protease K or endopeptidase K, is commonly used in molecular biology for protein digestion. The *H. speciosa* SE fraction latex of one experimental group was treated with proteinase K, according to the manufacturer's instructions (Sigma-Aldrich, r P2308).

Experimental groups

This study used 48 fertilized chicken eggs (Gallus domesticus). The chicken eggs were divided into four experimental groups containing 12 eggs each. The experimental groups were: (1) *H. speciosa* serum fraction (SE group); (2) inactivated protein of *H. speciosa* serum fraction latex (IPSE group; latex treated with proteinase K); (3) water (WA, negative control); (4) dexamethasone (DE, angiogenesis inhibitor).

Angiogenic potential and CAM assay

This study was approved by the Ethics Committee for the use of animals at the State University of Goiás, Brazil (Protocol no007/2018). A CAM assay with few modifications was performed as described by Almeida et al. (2014). The eggs were incubated at 37°C in a humidified environment (around 70% humidity). After five days of incubation, the CAM was accessed through a window cut in the eggshell, and the eggs were returned to the incubator. On day 13 of incubation, the CAM was exposed to the different treatments. Filter paper disks with 3 µl of solutions were used in each treatment. After exposing the CAM to different treatments, the eggs returned to the incubator for 72 hours. Then, the CAM was fixed in formaldehyde (3.7%) for 5 minutes. Scissors were used to remove the CAM from the egg and the CAM was maintained in Petri dishes with a formaldehyde solution. The newly-formed vascular net was analyzed and quantified through images captured with two different software: Gimp (version 2.0.5) was used to normalize the saturation, light, and contrast of the CAM images; and ImageJ (NIH, version 1.28) was used to quantify the number of pixels in each image. The angiogenic activity of the serum fraction latex was evaluated by comparing the treated and control groups with one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test to compare the treatment means. A p-value <0.05 was used to indicate statistical significance.

After analyzing the images, the CAM was embedded in paraffin for histological analysis, stained with hematoxylin-eosin, and examined by optical microscopy. The parameters evaluated were conjunctive cells, inflammation, and neovascularization. The results were classified based on their intensity, and the data were transformed into quantitative variables by assigning the following scores: 0 for absent (0%), 1 for discrete (1-25%), 2 for moderate (26-50%), and 3 for accentuated (over 51%). The results were analyzed with the Kruskal-Wallis test at a significance level of $p<0.05$, followed by Dunn's multiple comparisons.

Total protein quantification

The total of proteins in the serum fraction latex was determined with the Bradford method with modifications (Bradford 1976). For this test, the standard curve was first prepared with albumin (1 mg/mL BSA) and its serial dilutions in H2O (0.5 mg/mL, 0.25 mg/mL, and 0.125 mg/mL). The serum fraction latex was prepared with 2.37 mL of water, 30 µL of serum fraction latex, and 600 µL of Bradford. The mixture was incubated for 5 minutes in the dark and measured at 595 nm (SpectraMax Paradigm, Molecular Devices). The same protocol was used for measuring the total protein in the *H. brasiliensis* serum latex.

Enzyme activity quantification

The protease activity was determined with casein as a substrate, according to the methodology by Sarath et al. (2001). The enzymatic activity was expressed in U/mL and defined as the number of enzymes needed to promote the release of 1 µmol of Tyr/min under testing conditions. The β-1,3-glucanase activity was determined with laminarin as a substrate (Noronha and Ulhoa 2000). The amount of reducing sugar was determined at 540 nm. One unit of enzymatic activity (U) was defined as the number of enzymes needed to

release 1 µmol of reducing sugar per minute. The N-acetylglucosaminidase (NAGase) activity was determined with N-acetyl-β-D-glucosaminide (Ulhoa and Perberdy 1992). One unit (U) of N-acetyl-β-D-glucosaminidase was defined as the number of enzymes needed to hydrolyze 1 µmol of p-nitrophenol per minute. Acid phosphatase was determined with a substrate derived from *p*-nitrophenyl-phosphate (2.5 mM), according to the methodology by Monteiro et al. (2015). One unit of phosphatase was defined as the number of enzymes required to produce 1 µmol of ρ-nitrophenol per minute. The beta-glucosidase activity was determined with a substrate derived from p-nitrophenyl-β-D-glucopyranoside (2.5 mM). An enzyme unit of N-acetyl-β-D-glucosidase was defined as the number of enzymes required to produce 1 µmol of p-nitrophenol per minute. Chitinase activity was determined with a substrate derived from *p*-nitrophenyl-N-N-diacetylchitobiose (2.5 mM), according to the methodology by Qualhato et al. (2013). A chitinase enzyme unit was defined as the number of enzymes required to produce 1 µmol of p-nitrophenol per minute. Lipase activity was performed with the p-nitrophenyl palmitate synthetic substrate, following the release of the p-nitrophenyl palmitate ion (410 nm). One enzyme unit (U) was defined as the number of enzymes needed to hydrolyze 1 µmol of the substrate in one minute (µmol/min) under testing conditions. To obtain a relative measure of enzyme quantification (Table 3), the specific activity (the number of units per milligram of protein) was calculated with the formula:

$$\frac{\text{enzyme activity (U)}}{\text{protein concentration (mg)}}$$

3. Results

To evaluate the role proteins of *H. speciosa* serum fraction latex in angiogenesis, the percentages of CAM vascular area in the treatment and control groups were calculated. Figure 1 shows the representative images of the vascular nets of each condition. The average percentage of vascularization obtained for each group was WA 14.03 ± 1.4, DE 9.30 ± 1.3, SE 26.55 ± 1.5, and IPSE 11.7 ± 2.4. The serum fraction, as expected, showed an increase in the percentage of vascularization relative to water (negative control), which confirms the angiogenic activity of the *H. speciosa* serum fraction latex. However, the serum fraction treated with proteinase K and without active proteins (IPSE group) did not show angiogenic activity. Vascularization in the IPSE group did not show significant differences in the percentage of vascularization with inhibitory control (DE).

The histological analyses evaluated three different parameters (Figure 2). For inflammation, the serum fraction latex was significantly different from dexamethasone (Table 1). Regarding the conjunctive cells, the serum fraction latex group was significantly different from dexamethasone and water (Table 1). As for angiogenesis, the serum fraction group was significantly different from all other groups (WA, DE, and IPSE), showing an increase in angiogenesis (Table 1). The histological results agree with the morphological data, indicating that the *H. speciosa* serum fraction latex presents angiogenic activity. However, this biological property is lost after the enzymatic treatment with proteinase K.

Table 1. Histological parameters of the chorioallantoic membranes treated with the control solutions (W: water and DE: dexamethasone) and *H. speciosa* serum fractions (SE: serum latex; IPSE: inactivated proteins serum latex using Proteinase K). The results were classified based on their intensity, and the data were transformed into quantitative variables by assigning the following scores: 0 for absent, 1 for discrete, 2 for moderate, and 3 for accentuated.

Samples	Inflammation		Conjunctive cells		Angiogenesis	
	Median	Bonferroni test	Median	Bonferroni test	Median	Bonferroni test
W	1	A	0	A	0	A
DE	0	AB	0	AC	0	A
SE	2	AC	3	BD	2,5	B
IPSE	1	A	1	A	0	A
	P=0,005		*P=0,003*		*P=0,003*	

* The data were submitted to non-parametric Kruskal-Wallis analysis. Same letters represent no significant difference by the Bonferroni t-test.

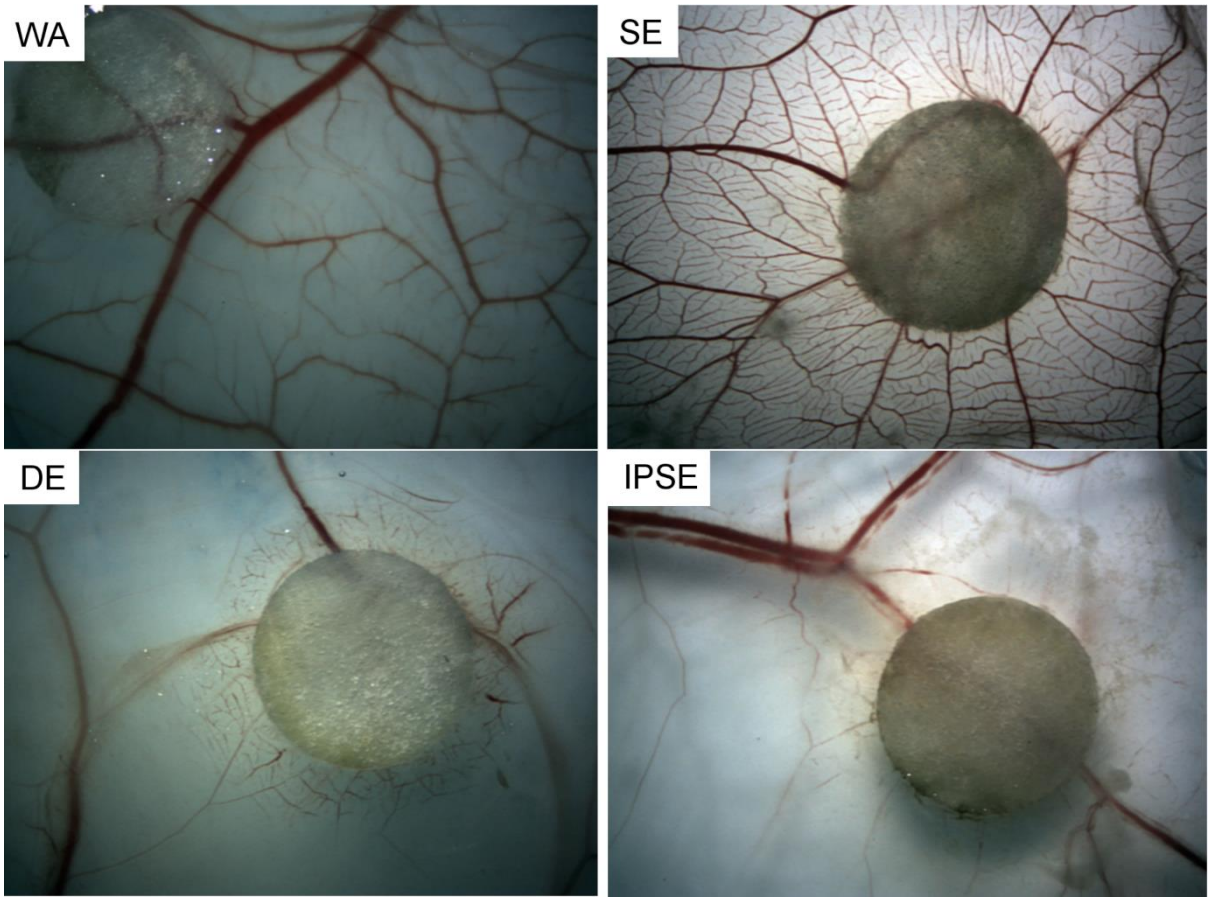

Figure 1. Angiogenic potential of the *Hancornia speciosa* latex on the chorioallantoic membrane assay (CAM). Representative images of different CAM treatments: WA (water, negative control); DE (dexamethasone, angiogenesis inhibitor); SE (*H. speciosa* serum fraction); IPSE (*H. speciosa* serum fraction after inactivation with proteinase K).

To evaluate the protein content in the *H. speciosa* serum fraction latex, the Bradford method was used. The results obtained were compared with the values observed in the *Hevea brasiliensis* serum fraction latex. *H. brasiliensis* was used as the control because its latex is established in the literature. The protein content found in the *H. speciosa* serum fraction was 0.17 mg/mL, while *H. brasiliensis* showed 1.1 mg/mL. Tables 2 and 3 show the non-specific and specific enzymatic activities of the serum fraction latex of both species, respectively. As a result, there was no enzyme activity for NAGase, phosphatase, and chitinase.

Lipase showed very low activity, while β-glucosidase and proteases showed significant activity, and a remarkable activity of β-1,3-glucanase was detected in the *H. speciosa* serum fraction.

Table 2. Enzymatic activity of *Hancornia speciosa* SE fraction and in *H. brasiliensis* SE fraction. The enzymatic activity was expressed in U/mL.

Samples	Protease	β-1,3 glucanase	NAGase	Phosphatase	β-glucosidase	Chitinase	Lipase
H. speciosa SE fraction	1.95	92.05	0	0	2.45	0	0.2
H. brasiliensis SE fraction	8.37	5.35	5.92	8.54	7.02	4.73	2.52

Table 3. Specific enzymatic activity of *Hancornia speciosa* SE fraction and in *H. brasiliensis* SE fraction. The enzymatic activity was expressed in U/mL.

Samples	Protease	β-1,3 glucanase	NAGase	Phosphatase	β-glucosidase	Chitinase	Lipase
H. speciosa SE fraction	11.47	541,47	0	0	14.41	0	1.18
H. brasiliensis SE fraction	7.61	4.86	5.38	7.76	6.38	4.30	2.29

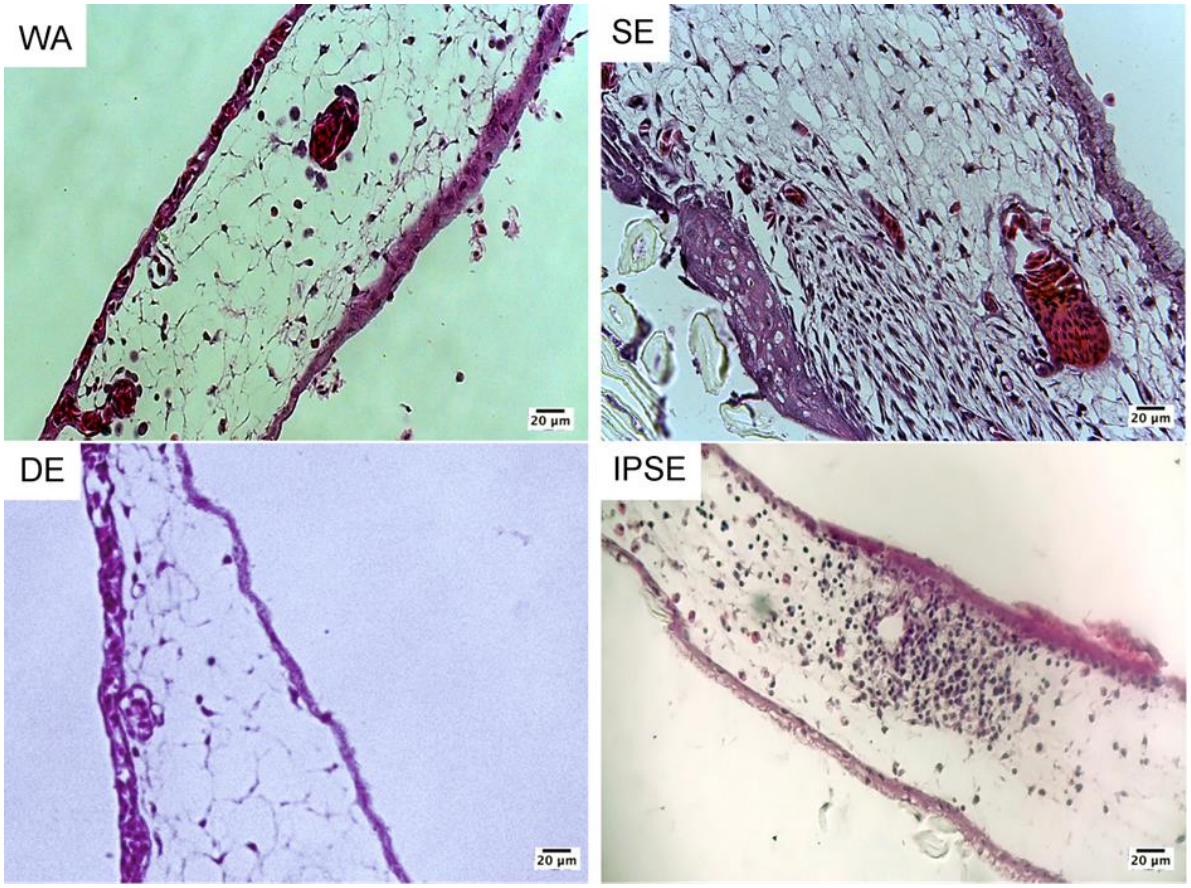

Figure 2. Histological analysis of chorioallantoic membranes (CAM) treated with *Hancornia speciosa* SE fractions. Paraffin sections stained with hematoxylin-eosin for different groups: WA (water, negative control); DE (dexamethasone, angiogenesis inhibitor); SE (*H. speciosa* serum fraction); IPSE (inactivated proteins serum latex of *H. speciosa* using proteinase K).

4. Discussion

The tissue repair process is extremely complex and begins immediately after the injury (Campos et al. 2007). This process is initially characterized by vascular and cellular events such as the increase in vascular permeability and angiogenesis (Mendonça and Coutinho-Netto 2009). This study evaluates the activity of proteins in the *H. speciosa* latex in angiogenesis, using the CAM assay. The results obtained may help to identify the agents that control angiogenesis and which of them can benefit the development of therapeutic drugs for tissue regeneration.

The CAM assay results confirm the angiogenic activity of the *H. speciosa* serum fraction latex (Figure 1). Additionally, the lack of an angiogenesis process in the IPSE group, which was treated with proteinase K, confirms that the stimulation of angiogenesis in the CAM membrane is associated with proteins in the serum fraction.

As the proteins showed an important role in angiogenesis, the total protein content in the serum fraction and its enzymatic activity were investigated. There was a low protein content in the *H. speciosa* serum fraction latex. This was previously observed by Malmonge et al. (2009), who identified a low protein concentration in the *H. speciosa* crude latex (1,900 $\pm$ 32 µg/g dw). The literature shows that proteins are the main responsible for latex allergies (Kelly and Sussman 2017). Our results suggest that the *H. speciosa* latex could be used to develop new drugs with a hypoallergenic response.

Enzyme activity expressed only per unit volume or mass may not be a relevant factor, as discussed by Bisswanger (2014). The present study showed a low soluble protein for the *H. speciosa* SE fraction but indicated angiogenesis activity. This study also used the relationship between non-specific enzymatic activity and the amount of protein found in the samples, called specific activity.

Despite the low protein concentration compared to other lactiferous, it was possible to detect significant non-specific (U/mL) and specific (U/mg protein) enzymatic activities in the *H. speciosa* serum

fraction. Three enzymes showed a significant non-specific activity: β-1,3 glucanase, β-glucosidase, and proteases (Table 2). When correlating the total enzyme activity to total protein, the specific enzymatic activity in *H. speciosa* SE fraction significantly increased for those enzymes compared with the *H. brasiliensis* SE latex (Table 3).

The help from these enzymes in the wound healing process must be understood. The 1,3-β-glucanases are fibrolytic enzymes that participate in the hydrolysis of carbohydrates for energy production in fungi and bacteria (Usoltseva et al. 2020) and as a response after infections by plant pathogens (Kim and Hwang 1994; Hong and Meng 2003). Because of the fibrolytic activity, this enzyme could assist debridement and remodeling in the physiological healing process. During the remodeling process, the fibroblast produces the fundamental amorphous substance (mucopolysaccharides) involved in the production, size, and orientation of collagen fibers (Junqueira and Carneiro 2017), and the 1,3-β-glucanases could help tissue organization. Besides helping the orientation of collagen fibers, it was suggested that 1,3-β-D-glucanases could promote cell proliferation (Jose et al. 2014). Another possible effect of 1,3-β-D-glucanases on the wound healing process is antifungal activity (Usoltseva et al. 2020; Hong and Meng 2003), which could reduce the chances of wound contamination. Regarding the toxic potential, there was no cytotoxic effect when exposing animal cells to 1,3-β-D-glucanases, even in high concentrations (Usoltseva et al. 2020).

The β-glucosidase catalyzes the hydrolysis of glycosidic bonds to terminal non-reducing residues in beta-D-glucosides and oligosaccharides, releasing glucose (Cox et al. 2000). The β-glucosidase could be important to destroy the connective tissue matrix in the inflammatory process (Chithra et al. 1998). Additionally, these enzymes could reduce oxidative damage (Romero-Segura et al. 2012). Aloe vera is an example of a plant species used for wound healing that contains β-glucosidase activity in its extract (Chithra et al. 1998).

Regarding protease activity, some studies have shown that these enzymes may have clot-inducing and hydrolyzing properties (Urs et al. 2017). Clot formation is vital for hemostasis, which is the initial phase of wound healing when proteases present procoagulant and thrombin-like activities. Also, proteases present fibrinolytic, gelatinolytic, and collagenolytic activities, which may aid wound debridement (Urs et al. 2017).

Proteases usually offer excellent conditions for physiological wound healing, considering they complement endogenous proteases in hemostasis, attenuate microbial activity, debride wounds, and stimulate angiogenesis and cell proliferation (Urs et al. 2017). Some examples of proteases used in the wound healing process are papain (Udod and Storozhuk 1979), bromelain (Maurer 2001), curcain (Nath and Dutta 1991), fibrinolysin (Cavalcanti et al. 2012), and ficain (Shiksharthi and Mittal 2001; Devaraj et al. 2008).

These enzymes could influence the healing process, aiding the debridement and remodeling of the extracellular matrix and collagen deposit, and decreasing the chances of contamination by microorganisms. Although the results obtained with the CAM model show a high degree of compliance with the results obtained in mammalian trials, further studies should be performed to evaluate the effects of the *H. speciosa* latex enzyme on the wound healing process.

5. Conclusions

The proteins in the *H*. speciosa serum fraction latex present angiogenic activity and can be beneficial to the development of therapeutic materials for wound healing and tissue regeneration. The protein content of the serum fraction is low compared to other latex. Despite the low concentration, it was possible to detect significant non-specific and specific enzymatic activities of β-1,3 glucanase, β-glucosidase, and proteases in this biomaterial. These enzymatic activities could assist the wound healing process and be useful to develop new drugs.

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Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: This study was approved by the Ethics Committee for the use of animals at the State University of Goiás, Brazil (Protocol number 007/2018).

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References

ADAIR, T.H. and MONTANI, J.P. Angiogenesis. San Rafael (CA): Morgan & Claypool Life Sciences, 2010. https://doi.org/10.4199/c00017ed1v01y201009isp009

ALEKSANDROWICZ, E. and HERR, I. Ethical euthanasia and short-term anesthesia of chick embryo. *Alternative to Animal Experimentation* (ALTEX). 2015, **32**(2), 143-147. https://doi.org/10.14573/altex.1410031

ALMEIDA, L.M., et al. *Hancornia speciosa* latex for biomedical applications: physical and chemical properties, biocompatibility assessment and angiogenic activity. *Journal of Materials Science: Material in Medicine.* 2014, **25**(9), 2153-2162. https://doi.org/10.1007/s10856-014-5255-8

ARRUDA, A.S., et al. Mangabeira latex production evaluation in Cerrado region of Goiás. *Ciência Florestal*. 2016, **26**(3), 939-948. https://doi.org/10.5902/1980509824222

BISSWANGER, H. Enzyme Assays. *Perspectives in Science*. 2014, **1**(1-6), 41-55. https://doi.org/10.1016/j.pisc.2014.02.005

BONETE, J.M., et al. Tissue reaction and anti-biofilm action of new biomaterial composed of latex from *Hancornia speciosa* Gomes and silver nanoparticles. *Anais da Academia Brasileira Ciências.* 2020, **16**(92), e20191584. https://doi.org/10.1590/0001-3765202020191584

BRADFORD M.M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-Dye Binding. *Analytical Biochemistry*. 1976, **72**(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

CAMPOS, A.C.L., BORGES-BRANCO, A. and GROTH, A. Wound healing. *Arquivos Brasileiros de Cirurgia Digestiva*. 2007, **20**(1), 51-58. https://doi.org/10.1590/S0102-67202007000100010

CAVALCANTI, J.M., et al. The essential oil of Croton zehntneri and trans-anethole improves cutaneous wound healing. *Journal of Ethnopharmacology*. 2012, **144**(2), 240-247. https://doi.org/10.1016/j.jep.2012.08.030

CHITHRA, P., SAJITHLAL, G.B. and CHANDRAKASAN, G. Influence of *Aloe vera* on the glycosaminoglycans in the matrix of healing dermal wounds in rats. *Journal of Ethnopharmacology*. 1998, **59**(3), 179-186. https://doi.org/10.1016/S0378-8741(97)00112-8

COSTA, A.F., et al. *Hancornia speciosa* serum latex fraction: a non-allergenic biomaterial. *Brazilian Journal of Biology*. 2023, **83**, e251075. https://doi.org/10.1590/1519-6984.251075

COX, M.M., LEHNINGER, A.L. and NELSON, D.L. Lehninger principles of biochemistry. 3th ed. New York: Worth Publishers. 2000. pp. 306–308.

D'ABADIA, P.L., et al. *Hancornia speciosa* serum fraction latex stimulates the angiogenesis and extracellular matrix remodeling processes. *Anais da Academia Brasileira de Ciências*. 2020, **92**(2), e20190107. https://doi.org/10.1590/0001-3765202020190107

DANCIU, C., et al. Evaluation of phenolic profile, antioxidant, and anticancer potential of two main representants of Zingiberaceae family against B164A5 murine melanoma cells. *Biological Research.* 2015, **48**, 1-9. https://doi.org/10.1186%2F0717-6287-48-1

DEVARAJ, K.B., GOWDA, L.R. and PRAKASH, V. An unusual thermostable aspartic protease from the latex of *Ficus racemosa* (L.). *Phytochemistry*. 2008, **69**(3), 647–655. https://doi.org/10.1016/j.phytochem.2007.09.003

DE ALMEIDA, L.M., et al. The state-of-art in angiogenic properties of latex from different plant species. *Current Angiogenesis*. 2015, **4**(1), 10-23. http://dx.doi.org/10.2174/2211552804011605171645 31

DO PRADO, A.D.L., et al. The chick embryo chorioallantoic membrane assay as a model for the study of angiogenesis. *Bioscience Journal*. 2019, **35**(4), 1262–1275. https://doi.org/10.14393/BJ-v35n4a2019-42777

DOS SANTOS NEVES, J., et al. Evaluation of osteogenic potential of *Hancornia speciosa* latex in rat calvaria and its phytochemical profile. *Journal of Ethnopharmacology*. 2016, **13**(183), 151-158. https://doi.org/10.1016/j.jep.2016.02.041

FLORIANO, J.F., et al. Comparative study of bone tissue accelerated regeneration by latex membranes from *Hevea brasiliensis* and *Hancornia speciosa. Biomedical Physics Engineering Express*. 2016, **2**(4), e045007. https://doi.org/10.1088/2057-1976/2/4/045007

FOLKMAN J. Fundamental concepts of the angiogenic process. *Current Molecular Medicine*. 2003, **3**(7), 643-651. https://doi.org/10.2174/1566524033479465

HONG, T.Y. and MENG, M. Biochemical characterization, and antifungal activity of an endo-1,3-beta-glucanase of *Paenibacillus* sp. isolated from garden soil. *Applied Microbiology Biotechnology*. 2003, **61**(5), 472-478. http://dx.doi.org/10.1007/s00253-003-1249-z

JOSE, D., et al. Potential application of beta-1,3 glucanase from an environmental isolate of *Pseudomonas aeruginosa* MCCB 123 in fungal DNA extraction. *Indian Journal of Experimental Biology*. 2014, **52**(1), 89-96.

JUNQUEIRA, L.C. and CARNEIRO, J. - Tecidos conjuntivos. In JUNQUEIRA, L.C.; CARNEIRO, J. Histologia Básica – Texto e Atlas. 13 ed. Rio de Janeiro. Guanabara Koogan. 2017. p 108-114.

KARIM, A.A., et al. Phenolic composition, antioxidant, anti-wrinkles and tyrosinase inhibitory activities of cocoa pod extract. *BMC Complement Alternative Medicine*. 2014, **14**, 381. http://dx.doi.org/10.1186/1472-6882-14-381

KELLY, K.J. and SUSSMAN, G. Latex allergy: where are we now and how did we get there? *The Journal of Allergy Clinical Immunology: In Practice.* 2017, **5**, 1212-1216. https://doi.org/10.1016/j.jaip.2017.05.029

KIM, Y.J. and HWANG, B.K. Differential accumulation of β-1,3 glucanase and chitinase isoforms in pepper stems infected by compatible and incompatible isolates of Phytophthora capsici. *Physiological and Molecular Plant Pathology.* 1994, **45**(3), 195-209. https://doi.org/10.1016/S0885-5765(05)80077-3

KONNO, K. Plant latex and other exudates as plant defense systems: roles of various defense chemicals and proteins contained therein. *Phytochemistry.* 2011, **72**(13), 1510-1530. https://doi.org/10.1016/j.phytochem.2011.02.016

MACEDO, M. and FERREIRA, A.R. Plantas medicinais usadas no tratamento dermatológico da Bacia do alto Paraguai, Mato Grosso. *Revista Brasileira de Farmacognosia*. 2004, **14**, 40-44. https://doi.org/10.1590/S0102-695X2004000300016

MALMONGE, J.A., et al. Comparative study on technological properties of latex and natural rubber from *Harconia speciosa* gomes and *Hevea brasiliensis*. *Journal of Applied Polymer Science*. 2009, **111**(6), 2986–2991. .http://dx.doi.org/10.1002/app.29316

MARINHO, D.G., et al. The latex obtained from *Hancornia speciosa* Gomes possesses anti-inflammatory activity. *Journal of Ethnopharmacology.* 2011, **35**(2), 530-537. https://doi.org/10.1016/j.jep.2011.03.059

MAURER, H. Bromelain: biochemistry, pharmacology and medical use. *Cellular and Molecular Life Sciences* CMLS. 2001, **58**(9), 1234–1245. https://doi.org/10.1007/pl00000936

MENDONÇA, R.J. and COUTINHO-NETTO, J. Aspectos celulares da cicatrização. *Anais Brasileiros de Dermatologia* 2009, **84**(3), 257-262. http://dx.doi.org/10.1590/S0365-05962009000300007

MONTEIRO, V.N., et al. Trichoderma reesei - Mycoparasitism against *Pythium ultimum* is coordinated by G-alpha Protein GNA1 Signaling. *Journal Microbial Biochemical Technology*. 2015, **7**(1), 001-007. http://dx.doi.org/10.4172/1948-5948.1000173

NATH, L.K. and DUTTA, S.K. Extraction and purification of curcain, a protease from the latex of Jatropha Curcas Linn. *Journal of Pharmacy and Pharmacology*. 1991, **43**(2), 111–114. https://doi.org/10.1111/j.2042-7158.1991.tb06642.x

NETO, G. and GUARIM-MORAIS, R.G. Medicinal plants resources in the Cerrado of Mato Grosso State, Brazil: a review. *Acta Botanica Brasileira.* 2003, **17**, 561-584. https://doi.org/10.1590/S0102-33062003000400009

NORONHA, E.F. and ULHOA, C.J. Characterization of a 29-KDa ß-1,3-glucanase from *Trichoderma harzianum*. FEMS Lett. 2000, **183**(1), 119-123. https://doi.org/10.1111/j.1574-6968.2000.tb08944.x

NOWAK-SLIWINSKA, P., SEGURA, T. and IRUELA-ARISPE, M. The chicken chorioallantoic membrane model in biology, medicine, and bioengineering. *Angiogenesis*. 2014, **17**(4), 779-804. https://doi.org/10.1007/s10456-014-9440-7

PENHAVEL, M.V.C., et al. Efeito do gel da seiva do látex da Hevea brasiliensis na cicatrização de lesões cutâneas agudas induzidas no dorso de ratos. *Revista do Colégio Brasileiro de Cirurgia*. 2016, **43**(1), 48-53. https://doi.org/10.1590/0100-69912016001010

POTT, A. and POTT, V.J. 1994. Plantas do pantanal. EMBRAPA, Planaltina, 320 p.

QUALHATO, T.F., et al. Mycoparasitism studies of *Trichoderma* species against three phytopathogenic fungi: valuation of antagonism and hydrolytic enzyme production. *Biotechnology Letters*. 2013, **35,** 1461–1468. https://doi.org/10.1007/s10529-013-1225-3

RIBEIRO, T.P., et al. Evaluation of cytotoxicity and genotoxicity of *Hancornia speciosa* latex in Allium cepa root model. *Brazilian Journal of Biology.* 2016, **76**(1), 245-249. https://doi.org/10.1590/1519-6984.20114

SAMPAIO, T.S. and NOGUEIRA, P.C.L. Volatile componentes of mangaba fruit (*Hancornia speciosa* Gomes) at three stages of maturity. *Food Chemistry.* 2006, **95**(4), 606-610. http://dx.doi.org/10.1016/j.foodchem.2005.01.038

SARATH, G.; ZEECE, M.G. and PENHEITER, A.R. In Protease assay methods. Proteolytic enzymes: a practical approach, (Beynon, R. and Bond J.S. ed.), Oxford University Press, New York, NY pp. 45-76, 2001.

SHIKSHARTHI, A.R. and MITTAL, S. *Ficus racemosa*: Phytochemistry, traditional uses and pharmacological properties: a review. *International Journal of Recent Advances in Pharmaceutical Research.* 2001, **4**, 6-15.

SINGER, A.J. and CLARK, R.A. Cutaneous wound healing. *The New England Journal of Medicine*. 1999, **2**(341), 738-746. https://doi.org/10.1056/nejm199909023411006

UDOD, V. and STOROZHUK, V. Treatment of suppurative diseases of soft tissues with proteolytic enzyme, papain. *Klinichna khirurhiia.* 1979, **1**, 37–38.

ULHOA, C.J. and PEBERDY, J.F. Purification and some properties of the extracellular chitinase produced by *Trichoderma harzianum*. *Enzyme and Microbial Technology*. 1992, **14**(3), 236-240. https://doi.org/10.1016/0141-0229(92)90072-V

URS, A. P., et al. Plant Latex Proteases: Natural Wound Healers. *In Proteases in Physiology and Pathology*. 2017, 297-323. http://dx.doi.org/10.1007/978-981-10-2513-6_14

USOLTSEVA, R.V., et al. Laminarans and 1, 3-β-D-glucanases. *International Journal of Biological Macromolecules.* 2020, **15**(163), 1010-1025. https://doi.org/10.1016/j.ijbiomac.2020.07.034

WITTENAUER, J., et al. Inhibitory effects of polyphenols from grape pomace extract on collagenase and elastase activity. *Fitoterapia*. 2015, **101,** 179-187. https://doi.org/10.1016/j.fitote.2015.01.005

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