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ORIGINAL ARTICLE

Chemical composition and antibacterial action of *Stryphnodendron pulcherrimum* bark extract, "barbatimão" species: Evaluation of its use as a topical agent

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KEYWORDS

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Acute dermal toxicity

Abstract *Stryphnodendron pulcherrimum* (Willd.) Hochr. (Fabaceae) is a native Amazonian tree, popularly known as "*barbatimão*". The stem barks of this tree are widely used in folk medicine to treat infections, cutaneous and ulcerative wounds, under the form of a suspension of the bark-derived powder. The present study aimed to investigate the *in vitro* antibacterial and cytotoxic activities of the ethanolic extract of *S. pulcherrimum* (SPEE). Likewise, to putatively identify the active compounds of the extract by ultra-high performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS). Therefore, we aimed to rationalize this observation by turning the bark powder-derived suspension into an ointment (SPEEO) incorporating SPEE (20% w/v). Both the antibacterial action and the acute dermal toxicity were evaluated in rats as animal model. Mortality, body weight changes, feed and water intake, organ weights, histological and biochemical parameters were measured for 14 days post-treatment with no major sign of toxicity. The SPEE showed excellent *in vitro* antibacterial activity (Minimum Inhibitory Concentration = 100 μg/m L) against *Staphylococcus aureus* and no *in vivo* acute dermal toxicity. The SPEEO inhibited up to 85% bacterial growth in a petri dish, with better results than the control standard ointment (Kollagenase®). We putatively identified 13 compounds present in the SPEE, belonging to the class of tannins, including one trimer and two dimers of catechin. Thereby, the data showed SPEEO as a potent antibacterial candidate for herbal medicine preparation.

1. Introduction

Antimicrobial Resistance (AMR) is a serious threat to the public health worldwide, affecting the efficacy of several treatments and the success of surgeries, increasing their costs and the mortality risk (O'Meara, 2020). All this caused by pathogens like *Staphylococcus aureus*, *Pseudomonas aeruginosa* and many *Escherichia coli* strains (Lee et al., 2020). The risen of multiple antibiotic resistant microorganisms are favored for overuse of antibiotics over the past decades (Lima et al., 2020).

Plants, in general, represent a major source of inspiration for new drugs, as shown on literature (Newman and Cragg, 2020) and as we discussed previously (Lautié et al., 2020). Thus, a systematization of the nature of the compounds present in plant species already described for their pharmacological properties is a rewarding task to prepare the drugs of tomorrow. In the last two decades, the interest in plants with a high tannin concentration, with antibacterial, antifungal, wound healing and anti-inflammatory activities increased (Alreshidi et al., 2020; Anttila et al., 2013; Carvalho et al., 2018; Holetz et al., 2005; Lima et al., 1998; Lopes et al., 2005; Muetzel and Becker, 2006; Okuda and Ito, 2011; Panizza et al., 1988; Sanches et al., 2005; Scalbert, 1991; Schofield et al., 2001; Morey et al., 2016). The antimicrobial properties of hydrolysable and condensed tannins have been the object of several studies (Lima et al., 2016; Reyes et al., 2017; Scalbert, 1991), suggesting the capacity of those types of compounds to sustain antibacterial activity against a large number of species.

Plants from the genus *Stryphnodendron* Mart. (Fabaceae), popularly known as "*barbatimão*", have been used for many years in the treatment of diseases associated to wounds and infections, in the Brazilian folk medicine. Native Brazilian people use the stem barks from these trees to prepare decocts, infusions, and tinctures, which are topically administrated for the treatment of uterine and vaginal disorders, urinary infections, skin lesions, ulcerative wounds, bruises, and inflammations (Henriques et al., 2016; Panizza et al., 1988). In the literature, several biological activities for *Stryphnodendron adstringens* (the most known species of "*barbatimão*") have been reported, including anti-inflammatory (Henriques et al., 2016), antioxidant (Baldivia et al., 2018; Sabino et al., 2018; Souza-Moreira et al., 2018), anti-ulcer (Audi et al., 1999; Martins et al., 2002) and healing properties, particularly, as mentioned above, skin wounds (Pellenz et al., 2019), anticarcinogenic (Souza-Moreira et al., 2018) and anti-parasitic (Holetz et al., 2005; Ishida et al., 2009; de Pinho et al., 2012; Santos et al., 2009; Souza et al., 2007).

The chemical composition of the *Stryphnodendron* spp. includes a variety of condensed tannins, principally oligomeric proanthocyanidins in their bark (Souza-Moreira et al., 2018). Despite the number of studies on chemical composition and antibacterial activity associated with some species of *Stryphnodendron*, neither the chemical composition nor the pharmacological properties of the *Stryphnodendron pulcherrimum* species have been described so far. To our knowledge, there is only one report describing 25 amazon plant extracts with antibacterial activity against *Enterococcus faecalis*, where *Stryphnodendron pulcherrimum* reported a promising activity against this bacterium (de Castilho et al., 2014).

To prioritize the tasks, we chose to search and describe the compounds supporting the pharmacological/therapeutic activity – if any - of an ointment formulated with an ethanolic extract of *Stryphnodendron pulcherrimum* stems bark. Indeed, as opposed to enteral administration, topic administration is easier to reach, as it does not include the fastidious general toxicity profiling.

Thus, the purpose of the present study is to identify the chemical constituents of the ethanolic extract of the stem barks of *Stryphnodendron pulcherrimum* (SPEE). We also evaluated its *in vitro* antibacterial and cytotoxic activities. Once the extracts were incorporated in an ointment (SPEEO), we determined its acute dermal toxicity and its ability to inhibit microorganisms in skin wounds in an animal model.

2. Experimental section

2.1. Plant material collection and extraction

Stryphnodendron pulcherrimum stem bark was collected at Belém (1°26′16.29″S, 48°26′29.32″W), temperature 24 °C and 85% relative humidity. The botanical identification was corroborated by Dr. Silvane Tavares Rodrigues from the Brazilian Agricultural Research Corporation (EMBRAPA) Eastern Amazon (Pará-Brazil). A specimen voucher was deposited in the IAN Herbarium, accession code 192817.

The plant material was washed with tap water and 0.1% NaClO aqueous solution, and then dried in a circulating air oven at 45 °C until constant weight. The bark was crushed using a knife mil obtaining 1.0 kg of a moderately fine powder (particle size 355 μm). The powder was macerated twice with 4 L of ethanol at room temperature with agitation for 24 h each time. The suspension was filtered through paper by gravity and the ethanol was evaporated in a rotary evaporator under reduced pressure. Finally, the extract was dried in an air circulating oven until constant weight, yielding 298.0 g of dry extract. A total of 10 mg of ethanolic extract were dissolved in 1 mL of H_2O:MeOH (2:8, v/v) and filtered through a 50 mg C18 cartridge Solid Phase Extraction (SPE, Waters Inc, Milford, Ma, USA) which was preconditioned with 1 mL methanol and 1 mL of water. The filtrated was dried under vacuum and weighted. The solid residue was dissolved in 1 mL of methanol and filtered through a 0.45 μm hydrophilic filter (Millipore, Merk, Darmstadt, Germany), then diluted to a 100 μg/mL concentration. This sample was used for the subsequent UHPLC-HRMS analysis.

2.2. Condensed tannins analysis

For the condensed tannins quantification, the method Butanol-HCl (Julkunen-Tiitto, 1985) was used. After heating at 95 °C for 120 min the absorbance of the reaction mixture was measured at 530 nm. The results were expressed in mg equivalent of cyanidin per g of dry extract (mg ECya/g DE).

2.3. Liquid chromatography and mass spectrometry analysis

The analysis was performed on a UHPLC chromatography coupled to a Xevo G2-STof mass spectrometer (Waters Corp., Milford, MA, USA), equipped with an electrospray ionization (ESI) probe operating in negative ionization mode. The mass range was 100–1200 Da, and Leucine-enkephalin was used as a LockSpray reference compound. The source temperature was set to 120 °C with a cone gas flow of 50 L/h. The desolvation gas flow was set to 600 L/h at a temperature of 250 °C. The capillary and cone voltages were set to 3.0 kV and 40 V, respectively. MassLynx software (Waters) was used for both, system control and data acquisition.

The samples were analyzed in a BEH C18 column (Waters, 50 × 2.1 mm, 1.7 μm) using ultra-pure water (solvent A) and methanol (solvent B). The gradient was set as follows: 5–95% B in 12 min, followed by 5 min post-run at initial conditions for equilibration of the column. The flow rate was 0.3 mL/ min, and the injection volume was 5 μL.

2.4. Putative identification of compounds

An extensive bibliographic revision was performed for all the compounds and their spectrometric data previously reported in species of the genus *Stryphnodendron*. After a careful evaluation of the spectrometric data obtained for the *Stryphnodendron pulcherrimum* bark ethanolic extract, several compounds were putatively identified in negative ionization mode (based on MS/MS). To confirm the putative identification of the proposed phenolic derivatives, individual MS/MS spectra were consulted against the GNPS (https://gnps.ucsd.edu/ProteoSAFe/static/gnps-splash.jsp?redirect=auth) and MoNA (https://mona.fiehnlab.ucdavis.edu/) spectral databases.

2.5. Antibacterial testing

Antibacterial activity was evaluated against the following standard strains: (i) Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538) (ii) bacteria Gram-negative: *Pseudomonas aeruginosa* (ATCC 25853) and *Escherichia coli* (ATCC 8739). All samples were obtained from INCQS/FIOCRUZ (National Institute of Quality Control in Health, Brazil). All bacteria were previously seeded in Petri plates containing Mueller Hinton agar (Merck, Germany) and incubated at 37 °C for 24 h. For bacterial inoculum preparation, strains were grown to exponential phase in Mueller Hinton broth (MHB) (Merck, Germany) at 37 °C for 24 h and adjusted by diluting fresh cultures to a turbidity equivalent to 0.5 McFarland scale (approximately 2×10^8 CFU/mL) and then diluted until 1×10^3 CFU/mL, as adapted (Matos Lopes et al., 2015) and described according to the Clinical and Laboratory Standard Institute (CLSI, 2018). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays were performed using the broth microdilution method in MHB (National Committee for Clinical Laboratory Standards) (CLSI, 2018). MIC was defined as the lowest concentration of the extract with no visible growth of the microorganism in the resazurin colorimetric assay (Monteiro et al., 2012).

To determine the MIC, the extracts were dissolved in distilled water at the highest concentration of 1 mg/mL to the lowest of 0.01 mg/mL. The concentrations were always diluted in half. 100 μL of the extract were placed in each well of the microplate together with 100 μL of the bacterial inoculum ($1x10^3$ CFU/mL). After incubation, the development of a purple-pink color was considered as indicative of bacterial growth. Therefore, MIC was read as the lowest concentration of the extract where the purple-pink color was not observed. To determine MBC, 10 μL of broth was taken from each well and incubated in Mueller Hinton agar at 37 °C for 24 h for each bacterium. The MBC was defined as the lowest extract concentration that resulted in a colony count lower than three colonies per mL (99.9% killing) or no bacterial growth (de Quadros et al., 2011). Each test was performed in three replicates. The negative control consisted of 100 μL of the bacterial inoculum and 100 μL of DMSO. Chloramphenicol (50 μg/mL) and gentamicin (10 μg/mL) were used as positive controls for Gram-positive and Gram-negative bacteria, respectively.

2.6. Cytotoxicity assay

To evaluate the cytotoxicity of the *Stryphnodendron pulcherrimum* ethanolic extract (SPEE), the cell viability test of the model cells MRC-5 fibroblasts was evaluated by 3-(4,5-dimethylthiazol-2yl)-2,5-bromide-diphenyl-tetrazolium (MTT) assay (Mosmann, 1983). To perform this assay, MRC-5 fibroblasts were seeded in 96-well polystyrene culture plates at a concentration of $1x10^4$ cells/mL in RPMI complete medium. After 24 h, the medium was removed, and the cells were treated with 100 µL of control solutions and extracts at concentrations of 125–7.8 µg/mL diluted in RPMI complete medium. The plate was incubated for 24 h at 37 °C with 5% CO_2. After this, the medium was withdrawn to add 100 µL of the MTT solution (0.5 mg/mL) in all wells. Thereafter, the MTT solution was withdrawn and 100 µL of pure DMSO was added to all wells, the plate was read at 550 nm. For the determination of Cytotoxic Concentration (CC_{50}), the absorbance was used, and a linear regression analysis was performed in the Prisma® program.

2.7. Selectivity index

The selectivity index (SI) was used as a parameter to evaluate the pharmacological potential of the samples with antibacterial activity in fibroblasts, which was calculated by the ratio Eq. (1) between the CC_{50} of MRC-5 fibroblasts and the inhibitory concentration 50% (IC_{50}) for *S. aureus* ("Test No. 402: Acute Dermal Toxicity | en | OECD," n.d.).

$$(IS)\ \frac{CC_{50}\ in\ MRC-5}{IC_{50}\ S.aureus} \quad (1)$$

For interpretation of the results of the selectivity index, it was considered that an SI greater than 1 indicates that the compound has a higher selectivity for the bacteria than for the fibroblast. An SI lower than 1 indicates that the extract has higher toxicity to eukaryotic cells. The higher the ratio, the more selective is the drug on the bacterium, consequently, it has less effect on the host cell of mammals (Almeida et al., 2014).

2.8. Animal model

Mus Musculus male and female Swiss mice weighing between 25 and 35 g were used. The animals were obtained from the Central Bioterium of the Biological Sciences Institute of the Federal University of Pará (UFPA). All animals were kept under controlled temperature conditions (23 ± 2 °C) and a light–dark cycle of 12 h. All animals had free access to pellets of food and water. This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the Brazilian National Council of Animal Experimentation (http://www.sbcal.org.br/) and the NIH Guidelines for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering and the experiments are reported in compliance with the arrive guidelines. The institutional Committee for Animal Ethics of Federal University of Pará/UFPA (CEUA, Protocol: 6884050817) approved all the procedures used in this study.

2.9. Formulation of the Stryphnodendron pulcherrimum ethanolic extract ointment (SPEEO)

The formulation proportions for the ointment in this study was taken from the National Formulary of the Brazilian Pharmacopoeia (Brasileira, 2019). Table S1 (*c.f.* Supporting Information) illustrates the constituents.

For manipulation of the ointments for the acute dermal toxicity, the components were adjusted to a sufficient quantity for 6 g of ointment, where 3 g of the crude extract of *S. pulcherrimum* plus 3 g of the ointment base were prepared, thus reaching a final concentration of 50%. The toxic dose used was 1000 mg/kg, according to OECD number 402 ("Test No. 402: Acute Dermal Toxicity | en | OECD," n.d.). The ointment used to assess antibacterial action was manipulated with the same composition, changing only to 20% SPEE concentration.

2.10. Acute dermal toxicity test of the ointment

This test was carried out based on the guidelines of the Organization for Economic Cooperation and Development – OECD, number 402 ("Test No. 402: Acute Dermal Toxicity | en | OECD," n.d.) and number 404 (Test No. 404 Acute Dermal Irrit., 2002). For this, mice were randomly allocated into two groups (n = 6 per group) by sex and treated with 1000 mg/kg of SPEEO or by the solvent of the ointment (negative control). All animals had the dorsal region shaved 24 h before the acute dermal toxicity test. On the second day, before the treatment, the absence of edema or erythema was checked. Then, the animals were treated with the SPEEO or base of ointment, after which the region was covered with gauze and non-irritating plastic adhesive. After the treatment, the general effects presented by the experimental animals were observed at the intervals: 30, 60, 90, 120, 180 and 240 min on the first day and once a day, always at the same time in the next 13 days (Draize et al., 1944). Edema and erythema were classified into scores according to the standard protocol of OECD 404 (Test No. 404 Acute Dermal Irrit., 2002) as shown in Table S2 (*c.f.* Supporting Information), and the degree of irritation was classified according to the Federal Hazardous Substances Act of the USA (Nale, 1962).

Mild erythema is almost imperceptible and severe erythema have the appearance in the flesh or eschar formation. Mild edema has a well-defined border, moderate has a border of approximately 1 mm, and severe has a border of more than 1 mm of exceeding the area of exposure of the product. The primary irritation index (PI) was calculated by the following Eq. (2), where: the total score represents the sum of the edema and erythema scores; the number of sites represents the number of mice tested and the number of days represents how many days the mice were observed (Draize et al., 1944).

$$(IP)\ \frac{Total\ score}{Number\ of\ sites \times Number\ of\ days} \quad (2)$$

Moreover, we evaluated scores for erythema, eschar formation, edema, cutaneous irritation, water and food consumption, body weight, and temperature on days 0, 7, and 14 of the treatment. After 14 days of observation, *Stryphnodendron pulcherrimum* extract ointment treated and control animals were anesthetized with xylazine (10 mg/kg) solution and ketamine (100 mg/kg) to collect blood samples by cardiac

puncture. The blood was collected in tubes containing EDTA K3 for evaluation of hematological parameters and in tubes containing separator gel to obtain serum for biochemical analyzes such as urea, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), triglycerides, uric acid, total proteins, and albumin, with fasting of 12 h before blood collection. The tissues and organs of the thoracic and abdominal cavities were examined and weighed. The experimental design is shown in Fig. 1 *c.f.* Supporting Information.

2.11. In vivo antibacterial action

The determination of the antibacterial action was carried out *in vivo* (Morton and Malone, 1972), male mice were divided into experimental groups (n = 3) and anesthetized with xylazine (10 mg/kg) solution and ketamine (100 mg/kg). After anesthesia, each animal was submitted to dorsal trichotomy with a biopsy punch (5 mm) and the cutaneous wound produced received antisepsis with alcohol 70%.

The material was collected on days 0, 7, and 14 after wound induction. Before the first application, the wounds were cleaned with sterile saline solution and the crust formed was separated with sterile forceps, the material was then collected from the wound bed with a sterile swab and subsequently placed in a sterile saline tube. The material was seeded in Petri dishes containing Nutrient Agar that was incubated in a bacteriological incubator at 37 °C for 24 h (Matos Lopes et al., 2015). Twenty-four hour later, the colony growth was evaluated, and colony-forming units were counted, followed by Gram staining and isolation by specific culture (Mannitol or MacConkey) (Matos Lopes et al., 2015).

Mice were divided into the following groups: positive control, negative control, and treatment. Each group contained 4 mice. Treatment started 24 h after surgery and was topically administered twice a day for 14 days. The positive control group received treatment with a standard curative ointment (Kollagenase® ointment) containing collagenase and chloramphenicol. The negative control group received the vehicle (Vaseline). The treatment group received the ointment with SPEEO 20%.

2.12. Hematological and biochemical parameters

The laboratory blood evaluation was adapted (Almeida et al., 2008). Twelve Swiss mice were used to assess biochemical serum levels (triglycerides, total protein, albumin, urea, alanine, and aspartate aminotransferase) and the determinations were performed on a WIENERLAB CM-200 analyzer (Wiener Lab Group, Rosario, Argentina) with WIENER kits for each specific parameter. Hematological parameters (blood count and platelet count) were determined by automated analyzer PE-6800VET PROKAN (Prokan Inc, Shenzen, People's Republic of China) with PROKAN kits specific to the device. The analyzes were performed following the manufacturers' recommendations.

2.13. Oxidative stress parameters

2.13.1. Determination of nitric oxide (NO) production

The nitrite (NO_2) was estimated colorimetrically on the 14th day after treatment. The assay is based on the reduction of nitrate to nitrite using the Griess method (Granger et al., 1996). Nitrite level was determined in 100 μL of homogenate samples of the main organs (heart, liver, kidney, and lung) incubated with an equal volume of the Griess reagent for 10 min at room temperature. The absorbance was measured at 550 nm and calculated from a standard curve with sodium nitrite expressed per μmol/mL.

2.13.2. Determination of lipid peroxidation

Lipid peroxidation was measured in the heart, liver, kidney, and lung 14 days after treatment with SPEEO as an indicator of oxidative stress, using the thiobarbituric acid-reactive substances (TBARS) assay (Kohn and Liversedge, 1944; Percario et al, 1994). Briefly, homogenate samples (100 μL) from the same organs (heart, liver, kidney, and lung) were mixed with 0.05 M trichloroacetic acid (TCA) and 0.67% thiobarbituric acid (TBA; Sigma-Aldrich, St. Louis, MO) in 2 M sodium sulfate, and heated in a water bath at 94 °C for 90 min. The chromogen formed was extracted in *n*-butanol and measured at 535 nm. An MDA standard solution was used to construct a standard curve against which unknown samples were plotted. Results were expressed as malondialdehyde equivalents in nmol/L.

2.13.3. Total evaluation of Trolox equivalent antioxidant capacity (TEAC)

The total antioxidant capacity (TAC) in heart, liver, kidney, and lung were evaluated 14 days after the treatment with SPEEO by Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; Sigma-Aldrich) equivalent antioxidant capacity assay (TEAC), which provides relevant information that may effectively describe the dynamic equilibrium between pro-oxidant and antioxidant compounds. In this assay, 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (Sigma Aldrich) was incubated with potassium persulphate (Sigma Aldrich) to produces $ABTS^{\cdot+}$, a green/blue chromophore. The inhibition of $ABTS^{\cdot+}$ formation by antioxidants in the samples were expressed as Trolox equivalents, determined at 740 nm using a calibration curve plotted with different concentrations of Trolox (Sigma Aldrich) (Miller et al., 1993; Re et al., 1999).

2.13.4. Glutathione (GSH) Levels

The levels of GSH, expressed by the amount of free thiol, were determined in the samples in homogenate samples from heart, liver, kidney, and lung 14 days after treatment with SPEEO through induction using Ellman's reagent (Ellman, 1959). This assay was based on the production of a yellow color when 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) is added to compounds containing sulfhydryl groups. The GSH concentration was determined using a standard curve constructed with different concentrations of GSH in the reduced form. The absorbance was recorded at 412 nm in a microplate reader (SpectraMax 250, Molecular Devices, Union City, CA, USA) and results were expressed in μmol/mL.

2.14. Statistical analysis

The results were analyzed by unpaired Student's t-tests using the Holm-Sidak method or One-way ANOVA followed by Dunnett's posthoc tests. All values were reported as mean ± S.D and the significance level adopted was $P < 0.5$.

3. Results

3.1. Characterization of the chemical compounds from ethanolic extract of Stryphnodendron pulcherrimum.

The total content of condensed tannins quantified in the ethanolic extract was 128.87 mg ECya/g DE, this value is within the range found in literature (Santos et al., 2006) using the same methodology, they evaluated the content of condensed tannins present in bark of the species *S. adstringens* and *S. polyphyllum*, their content varies from 87 to 621 mg ECya/g dry matter depending on the time of year. Considering this result, the mass spectrometry analysis of the ethanolic extract (chromatogram Fig. 1 *c.f.* Supporting Information) correlates with the type of compounds found. A total of thirteen compounds were putatively identified as tannins and phenolic acids derivatives (Table 1) by comparison with the data found in the literature and online spectral libraries (GNPS, MoNA).

The accurate mass and fragmentation patterns of the main compounds present in the chromatogram were extracted and submitted to dereplication. According to Table 1, six organic acids derivates were found (**1**–**5**, **12**), as well as six condensed tannins oligomers with B-type linkages (**7**–**11**, **13**) and one flavan-3-ol derivatives (**6**). Regarding phenolic acids, compounds **1** and **2** $[M-H]^-$ at *m/z* 169 were characterized as gallic acid and 3,4-dihydroxyphenyl glycol, respectively. For gallic acid, the fragment at *m/z* 125 corresponds to a neutral loss of carbon dioxide $[(MH)-CO_2]^-$, for its isomer, the fragments at *m/z* 151 and *m/z* 123 correspond to dehydration $[(MH)-H_2O]^-$ followed by loss of carbon monoxide $[(MH)-H_2O-CO_2]^-$. The compound **3** $[M-H]^-$ at *m/z* 137.0236 characterized as 4-hydroxybenzoic acid generated fragment at *m/z* 93, due to loss of carbon dioxide $[(M-H)-CO_2]^-$. The same loss was observed for compound **4** $[M-H]^-$ at *m/z* 153.0190 identified as gentisic acid. Compound **5** $[M-H]^-$ at *m/z* 121.0282 was defined as benzoic acid due to the characteristic fragment at *m/z* 93 referring to the loss of carbon monoxide $[(M-H)-CO]^-$. The sixth phenolic acid (compound **12**) $[M-H]^-$ at *m/z* 197.0456 was characterized as syringic acid and showed fragments at *m/z* 182 $[(MH)-C_8H_7O_5^{\cdot}]^-$ referring to the loss of methyl radical, at *m/z* 167 $[(M-H)-CH_2O]^-$ followed by *m/z* 123 $[(M-H)-CH_2O-CO_2]^{-\cdot}$ which is related to the loss of CO_2.

Compound **6** derived from flavan-3-ol $[M-H]^-$ at *m/z* 289.0707 was characterized as catechin. This compound has already been reported in other species of *Stryphnodendron* (Pellenz et al., 2018; Souza-Moreira et al., 2018). In addition, the fragmentation pattern was confirmed in GNPS, as well as in the literature (Callemien and Collin, 2008). Based on the HPLC chromatogram (210 nm) as well as on the TIC (chromatogram Fig. 2 *c.f.* Supporting Information), the

Table 1 Putative identified compounds in the ethanolic extract from the stem bark of *S. pucherrimum* by LC-HRMS.

Peak	RT (min)	MF	$[M-H]^-$		Products ion	Compound	Ref. MS^2	Species
			Exp. *m/z*	Δm ppm				
1	0.46	$C_7H_6O_5$	169.0127	5.91	125	gallic acid	(Pellenz et al., 2018; Souza-Moreira et al., 2018) VF-NPL-QEHF000559[a]	SA, SP, SR
2	0.86	$C_8H_{10}O_4$	169.0507	3.54	151, 123	3,4-dihydroxyphenyl glycol	CCMSLIB00000578290[b]	N/R
3	1.62	$C_7H_6O_3$	137.0236	2.18	93	4-hydroxybenzoic acid	CCMSLIB00000578301[b]	N/R
4	1.86	$C_7H_6O_4$	153.0190	1.30	109	gentisic acid	CCMSLIB00000578339[b]	N/R
5	2.06	$C_7H_6O_2$	121.0282	4.13	93	benzoic acid	FiehnHILIC002613[a]	N/R
6	2.47	$C_{15}H_{14}O_6$	289.0707	1.72	245, 205, 179	catechin	(Pellenz et al., 2018; Souza-Moreira et al., 2018) CCMSLIB00000081478[b]	SA, SR
7	2.91	$C_{30}H_{26}O_{13}$	593.1323	4.72	441, 425, 289	Procyanidin-prodelphinidin dimer (B type)	(Giffoni de Carvalho et al., 2020)	SA
8	3.32	$C_{45}H_{38}O_{19}$	881.1944	1.70	713, 593, 575, 557, 425, 407, 305, 289, 287, 271	(epi)catechin-(epi)catechin-(epi)gallocatechin I	(Olšovská et al., 2013)	N/R
9	3.43	$C_{45}H_{38}O_{19}$	881.1944	1.70	713, 593, 575, 557, 425, 407, 305, 289, 287, 271	(epi)catechin-(epi)catechin-(epi)gallocatechin II	(Olšovská et al., 2013)	N/R
10	3.59	$C_{30}H_{26}O_{13}$	593.1273	3.70	575, 557, 441, 425, 423, 407, 305, 289	Procyanidin-prodelphinidin dimer (B type)	(Giffoni de Carvalho et al., 2020)	SA
11	3.66	$C_{30}H_{26}O_{13}$	593.1273	3.70	575, 557, 441, 425, 423, 407, 305, 289	Procyanidin-prodelphinidin dimer (B type)	(Giffoni de Carvalho et al., 2020)	SA
12	4.01	$C_9H_{10}O_5$	197.0456	3.04	182, 167, 123	syringic acid	FiehnHILIC003000[a]	N/R
13	4.42	$C_{45}H_{38}O_{19}$	881.1944	1.70	713, 593, 575, 557, 425, 407, 305, 289, 287, 271	(epi)catechin-(epi)catechin-(epi)gallocatechin III	(Olšovská et al., 2013)	N/R

SA: *Stryphnodendron adstringens*; SP: *Stryphnodendron polyphyllum*; SR: *Stryphnodendron rotundifolium;* N/R: Not reported in *Stryphnodendron* spp.

[a] MoNA (https://mona.fiehnlab.ucdavis.edu/).

[b] GNPS (https://gnps.ucsd.edu/ProteoSAFe/static/gnps-splash.jsp).

predominance of six compounds is evident. After assessing the TIC, compounds **7**, **8**, **9**, **10**, **11** and **13** were identified, from which, three (**7**, **10**, **11**) correspond to Procyanidin-prodelphinidin dimer (B type) (*m/z* at 593), with different chromatographic behavior and similar losses according to their fragmentation patterns, suggesting they are isomers. In Fig. 1 it was shown, in the presented study the proposed pathways to explain the fragments observed for all dimers. Starting with two dehydrations at *m/z* 575 $[(M-H)-H_2O]^-$ and *m/z* 557 $[(M-H)-H_4O_2]^-$. Another route is through RDA fission followed by dehydration at *m/z* 441 $[(M-H)-C_8H_8O_3]^-$ and *m/z* 423 $[(M-H)-C_8H_{10}O_4]^-$, respectively. The same reaction should explain occurrence of product ion at *m/z* 425 $[(M-H)-C_8H_8O_4]^-$ from precursor ion at *m/z* 593. The dehydration in ring C could produce the fragment at *m/z* 407 $[(M-H)-C_8H_{10}O_5]^-$. In addition to these pathways, a common reaction in flavan-3-ol dimers is QMupper (Callemien and Collin, 2008), where there is a cleavage in the C-C bond. This explains the fragments at *m/z* 305 $[(M-H)-C_{15}H_{14}O_6]^-$ and *m/z* 289 $[(M-H)-C_{15}H_{14}O_7]^-$, which correspond to (epi)gallocatechin and (epi)catechin, respectively.

The other three heaviest compounds detected, **8**, **9** and **13** at *m/z* 881.1944, were putatively identified to be (epi)catechin-(epi)catechin-(epi)gallocatechin I, II and III, with a molecular formula of $C_{45}H_{38}O_{19}$. The difference for the dimers previously reported, is in one unit more than (epi) catechin and a pathway for fragmentation is shown on Fig. 2. The fragment at *m/z* 713 $[(M-H)-C_8H_8O_4]^-$ is produced by RDA fission, while the ions at *m/z* 593 $[(M-H)-C_{15}H_{12}O_6]^-$ and *m/z* 305 [(M-H)-C15H12O6]$^-$ by two consecutive QMupper, followed by dehydration at *m/z* 287 $[(M-H)-C_{15}H_{14}O_7]^-$. For the ion at *m/z* 593, it is possible to explain the fragments at *m/z* 425 $[(M-H)-C_8H_8O_4]^-$ and *m/z* 407 $[(M-H)-C_8H_{10}O_5]^-$ through RDA and dehydration mechanisms, respectively. Still about the ion at *m/z* 593, two fragments, at *m/z* 575 $[(M-H)-C_{15}H_{10}O_5]^-$ and *m/z* 557 $[(M-HM-H)-C_{15}H_8O_4]^-$ were observed, which should be explained by two dehydration reactions. Finally, the precursor ion generates two fragments by QMpper reaction and dehydration reactions at *m/z* 289 $[(M-H)-C_{30}H_{24}O_{13}]^-$ and *m/z* 271 $[(M-H)-C_{30}H_{26}O_{14}]^-$. The structure was based on the fragmentation pattern observed and in the literature for Prodelphinidins (Callemien and Collin, 2008).

3.2. SPEE promoted in vitro antibacterial activity

The inhibitory and bactericidal effects of the SPEE against the pathogens are shown in Table S3 (*c.f.* Supporting Information). The SPEE showed activity against the gram-

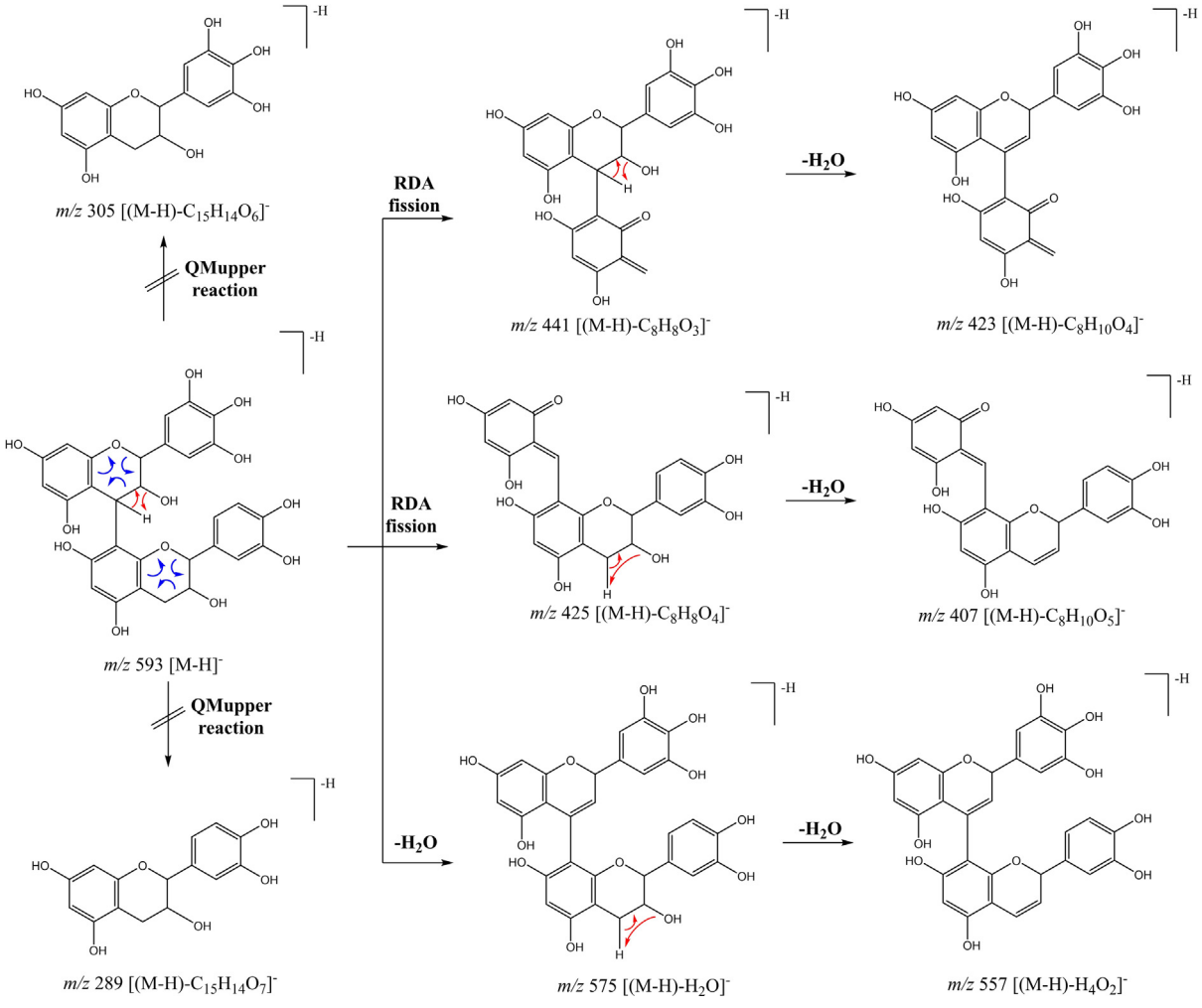

Fig. 1 Proposed fragmentation pathways for the major product ions observed in LC-MS/MS dimer isomers (**7**, **10** and **11**).

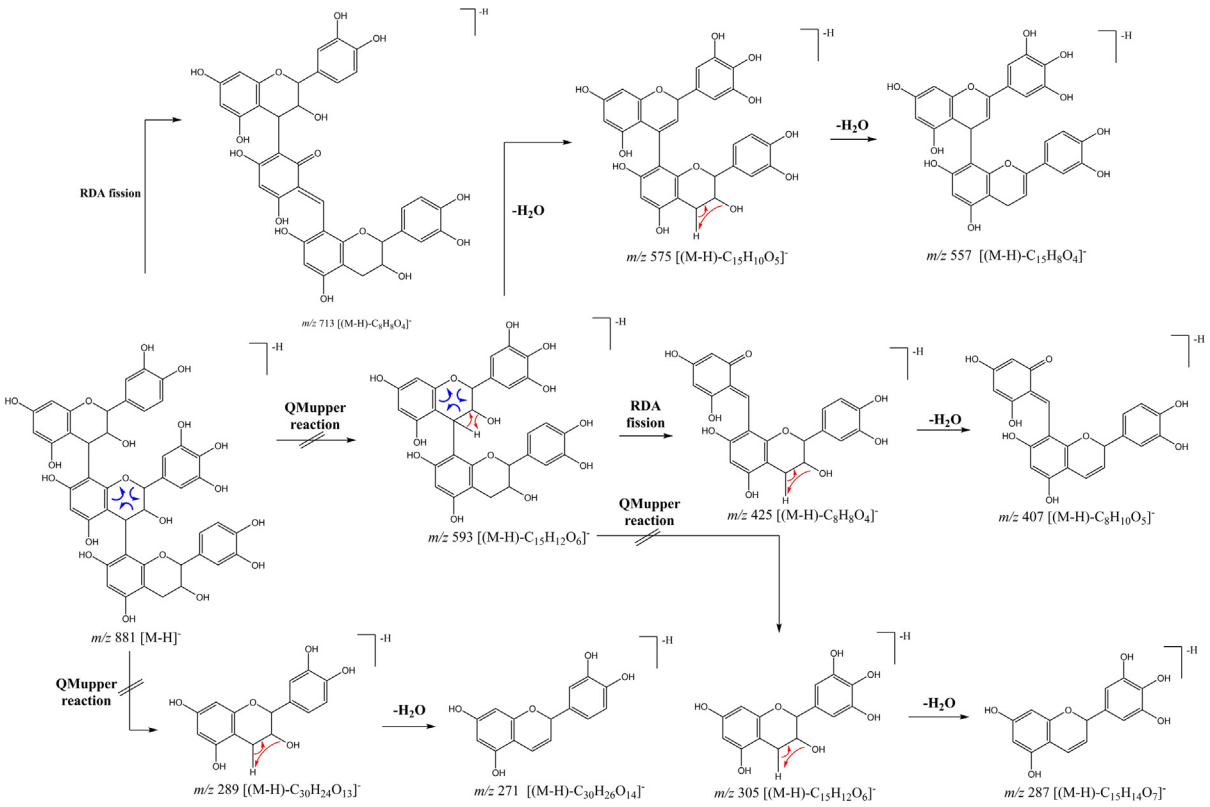

Fig. 2 Proposed fragmentation pathways for the major product ions observed in LC-MS/MS trimer isomers (**8**, **9** and **13**).

positive bacteria *S. aureus,* with a MIC value of 100 µg/mL and a MBC value of 200 µg/mL. Concerning gram-negative bacteria, the SPEE had MIC and MBC values of 900 µg/mL for *E. coli* and *P. aeruginosa*.

3.3. Cytotoxicity and selective index

In this study, the cytotoxicity of the SPEE was evaluated in MRC-5 fibroblasts. The data showed that the crude extract was able to kill 50% of the cells at a concentration of 40 µg/mL. The selectivity index of the SPEE was calculated based on the CC_{50} values in MRC-5 and IC_{50} for *S. aureus* bacteria that SPEE presented activity. Thus, the extract against *S. aureus* bacteria has a selectivity index of 0.44 showing lower selectivity to *S. aureus* (CC_{50} MRC-5 = 40 µg/mL and IC_{50} *S. aureus* = 90 µg/mL).

3.4. SPEE ointment shows no acute dermal toxicity test and clinical parameters.

The topical application of SPEEO (1000 mg/kg) was used to determine acute dermal toxicity in both sexes and was monitored for 14 days. Clinical parameters such as temperature, gain or loss of body weight, food consumption, water consumption, macroscopic toxicity, dermal irritation, adverse effects, or abnormal behavior were evaluated as general effects. No gross abnormalities were observed during necropsy for any of the animals at the 14-day observation point. All animals survived, no skin abnormalities such as erythema, scar formation, and edema were seen after treatment with 1000 mg/kg of SPEEO. The skin irritation index found for the animals was 0, so SPEE ointment is considered non-irritating to the skin. The SPEEO female group presented elevated temperature at day 0 compared to the sham group; however, they did not have alterations in the 4th and 14th days after treatment (Fig. 3A). The SPEEO male and female animals had normal body weight gain compared to sham animals (Fig. 3B).

Fig. 3C shows the mean food consumption daily of mice. All SPEEO groups had consumption like the sham one, although in the 14th day SPEEO females presented an increase in food intake compared to sham females (Fig. 3C). The water consumption was higher in SPEEO males compared to sham males on different days (4, 7, 12, and 13th day) (Fig. 3.D) and SPEEO female group consumed more in the 5 and 13th days.

3.5. SPEEO does not alter hematological parameters

The animals treated with SPEEO showed no significant changes in hematological parameters compared to sham animals in both sexes after 14 days of evaluation. However, platelet counts were lower in the group of male mice treated with SPEEO when compared to the sham group (Table 2).

3.6. SPEEO does not alter biochemical parameters

Overall, no significant biochemical changes in animals treated with SPEEO compared to control animals in both sexes, despite a mild augmentation of AST in SPEEO-treated male

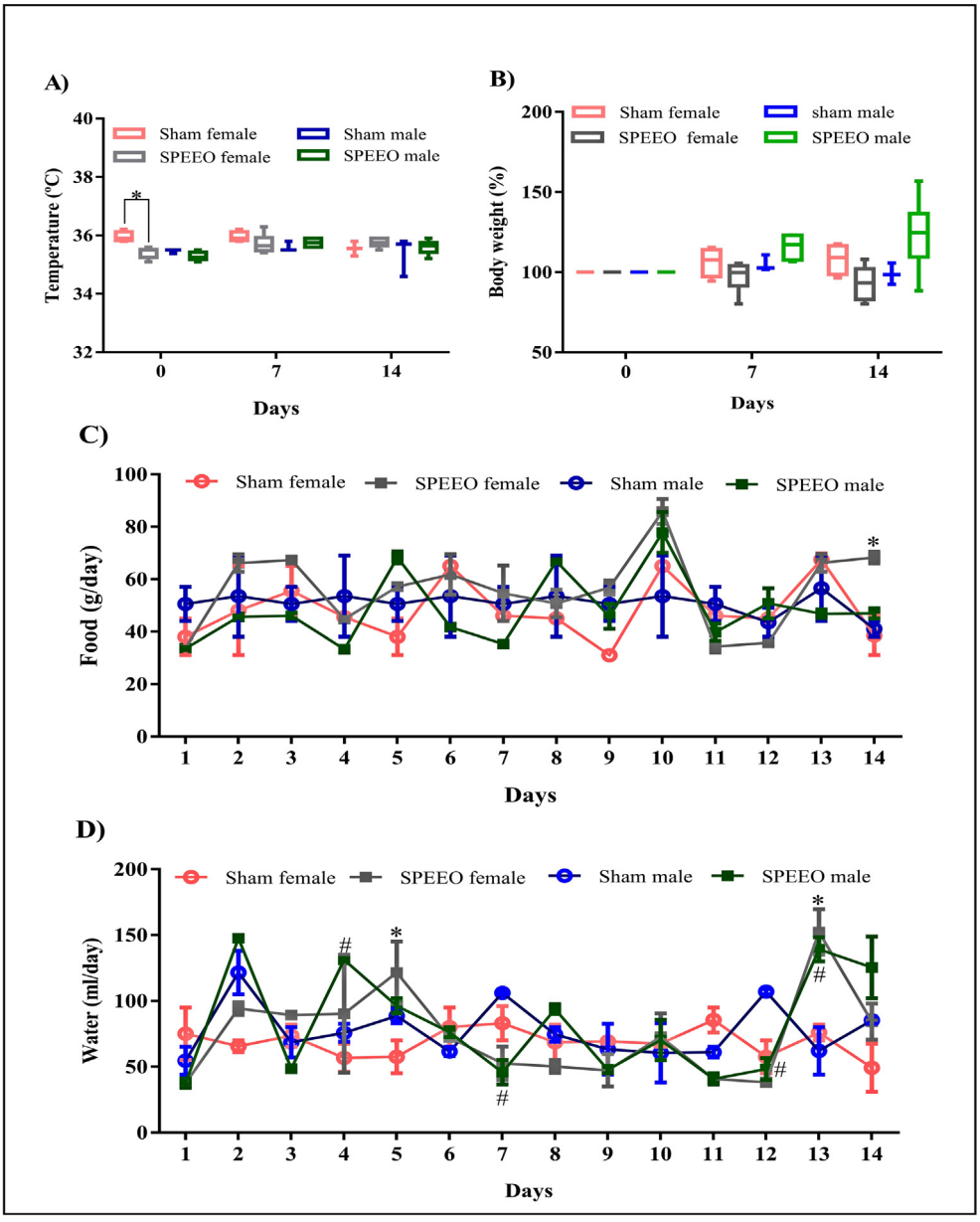

Fig. 3 Clinical parameters of mice treated with 1000 mg/kg of SPEEO or untreated during 14 days after treatment. Note: (A) temperature (B) body weight (C) food consumption (D) water consumption. Note: *Significant difference between sham female vs SPEE. O female ($p < 0.05$) # significant difference between sham male vs SPEEO male ($p < 0.05$). SPEEO: *S. pulcherrimum* ethanolic extract ointment.

animals compared to the sham group. Therefore, the data show that *S. pulcherrimum* extract at a dose of 1000 mg/kg did not cause physiological changes in the treated animals (Table 3).

3.7. SPEEO do not alter macroscopic organ rating and oxidative stress

Treatment with SPEEO did not change the relative weights of the organs of the animals compared to control animals in both sexes (Fig. 4A and B). Concerning the oxidative stress, assays did not show alterations in the organs of the animals, comparing groups treated with SPEEO or untreated. TBARS was not elevated, and the NO levels were maintained at similar levels in both treated and untreated groups (see Fig. 4C and D). Furthermore, the evaluation of antioxidant capacity by the TEAC method did not show a significant difference between the analyzed organs of the groups. These results demonstrate that baseline levels of TEAC and GSH are unchanged in the evaluated organs, both in treated and untreated groups (see Fig. 4E and F).

3.8. In vivo antibacterial action of SPEEO

Analyses of the antibacterial action of SPEEO showed a lower percentage of bacterial growth than Kollagenase® ointment

Table 2 Hematological values of mice treated with 1000 mg/kg of SPEEO or untreated.

Parameter (Units)	Female		Male		Reference Values[a]
	Sham	SPEEO	Sham	SPEEO	
Total RBC ($\times 10^6$/mm^3)	7.42 ± 0.11	7.53 ± 0.05	7.15 ± 0.49	6.39 ± 0.21	8.1 ± 1.0
Haemoglobin (g/dl)	11.26 ± 0.36	10.85 ± 0.35	11.30 ± 1.27	11 ± 1	12.1 ± 1.3
Haematocrit (%)	33.50 ± 0.70	33.50 ± 2.12	35.00 ± 5.65	31 ± 1	37.6 ± 3.6
MCV (fL)	45.05 ± 0.63	44.75 ± 0.35	49.05 ± 4.03	46.45 ± 2.05	46.7 ± 2.0
MCH (pg)	14.50 ± 0.70	14.45 ± 0.35	15.80 ± 0.70	15 ± 1	15.1 ± 1.1
MCHC (g/dL)	34.15 ± 1.20	32.40 ± 0.99	32.45 ± 1.62	33.650 ± 0.5	32.3 ± 2.7
Total WBC (/mm^3)	2850 ± 70	1750 ± 212	4300 ± 283	2501.50 ± 212	2500 ± 140
Eosinophils (/mm^3)	0 ± 0	0 ± 0	3 ± 2	0 ± 0	17.6 ± 1.5
Basophils (/mm^3)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	5.2 ± 3.7
Lymphocytes (/mm^3)	1515.50 ± 71.41	1178 ± 172	2713 ± 300	1601.50 ± 2.12	2164 ± 736
Monocytes (/mm^3)	59 ± 1	35 ± 4	36.2 ± 67.1	32.5 ± 3.5	18.5 ± 2.2
Banded neutrophils (/mm^3)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.0 ± 0
Segmented neutrophils (/mm^3)	522 ± 14	1234.00 ± 22.62	1056.50 ± 160.51	803 ± 4	341 ± 151
Platelet (/mm^3)	500000 ± 169705	439000 ± 1414	531500 ± 707	405000 ± 7071*	607000 ± 116

Note: Abbreviations: MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration, MCV: Mean Corpuscular Volume, RBC: Red Blood Cells, SPEEO: *S. pulcherrimum* ethanolic extract ointment, WBC: White Blood Cells. All the values are expressed as Mean ± SD (n = 6 in each group).

* Significant difference between SPEEO vs sham ($P < 0.001$).

[a] (Araújo, 2012; Santos et al., 2016.

Table 3 Effect of the acute dermal exposure of *S. pulcherrimum* on selected parameters (mean ± SD) in Swiss mice.

Parameter (Units)	Female		Male		Reference Values[a]
	Sham	SPEEO	Sham	SPEEO	
Urea (mg/dL)	58.67 ± 8.74	59.33 ± 6.11	51.33 ± 2.08	57.00 ± 2.00	44.90 ± 7.80
Creatinine (mg/dL)	0.24 ± 0.05	0.22 ± 0.03	0.24 ± 0.02	0.27 ± 0.02	0.30 ± 0.10
AST (U/L)	175.66 ± 44.37	160.33 ± 8.50*	119.00 ± 8.88	234.00 ± 52.14*	112.90 ± 31.50
ALT (U/L)	56.00 ± 16.09	51.33 ± 6.11	67.33 ± 2.51	57.66 ± 0.57	46.20 ± 14.30
Triglyceride (mg/dL)	99.33 ± 24.78	116.33 ± 55.32	103.00 ± 5.00	80.33 ± 2.30	130.00 ± 7.00
Total proteins (g/dL)	5.60 ± 0.51	5.33 ± 0.15	5.80 ± 0.26	4.87 ± 0.23	5.20 ± 0.60
Albumin (g/dL)	3.04 ± 0.10	3.13 ± 0.06	3.05 ± 0.21	2.87 ± 0.23	2.80 ± 0.10

Note: Abbreviation. ALT: alanine aminotransferase; AST: aspartate aminotransferase. SPEEO: *S. pulcherrimum* ethanolic extract ointment.

* Significant difference between SPEEO vs sham ($P < 0.001$).

[a] Araújo (2012) and Santos et al. (2016).

(control). After 7 days of treatment, the SPEEO was able to reduce the bacterial growth compared to the Kollagenase® ointment. On the14th day, the SPEEO inhibited 84.7% while the Kollagenase® ointment inhibited only 9.7%. Fig. 5 shows the percentage of bacterial growth at the 7th and 14th days, evidencing a marked difference between the control group and the SPEE ointment. Gram staining performed on samples collected from the mice wound bed showed the presence of gram-positive bacteria of the *Staphylococcus* genus.

4. Discussion

In the present study, the identification of the chemical compounds of the *S. pulcherrimum* ethanolic extract was carried out, as well as the quantification of its tannin content. In addition, we evaluated the acute dermal toxicity, the *in vitro* antibacterial activity, and its *in vivo* antibacterial action. The ethanolic extract showed a high content of tannins, mainly condensed tannins, which could be responsible for the excellent antibacterial effect against gram-positive bacteria such as *S. aureus*.

The SPEE showed greater *in vitro* inhibition capacity against *S. aureus* (MIC values of 100 µg/mL) than for *P. aeruginosa* and *E. coli* (MIC and MBC values of 900 µg/mL for both of them), thus showing low antimicrobial activity against gram-negative bacteria. (Machado et al., 2010) reported the antibacterial activity of tannins present in plant extracts have antimicrobial effect occurs for two main mechanisms of action: in gram positive they aggregate to the cell wall destroying the petideoglycan layer and the inhibition of protein synthesis. The first mechanism explains why the MIC for gram-negative bacteria is higher than gram-positive, since gram-negative bacteria have a thin layer of peptideoglycan but have an outer membrane that performs the function of providing an extra layer of protection and that acts as a barrier blocking the entry of molecules, which reduces the intracellular accumulation of the antibiotic by reducing cell permeability in addition to having resistance mechanisms such as the exclusion of antibiotics via the porin (Benucci et al., 2016; Delcour, 2009; Guo et al., 2018; Loureiro et al., 2016).

The antimicrobial activities of extracts from *Stryphnodendron* species against bacteria and fungi have been previously

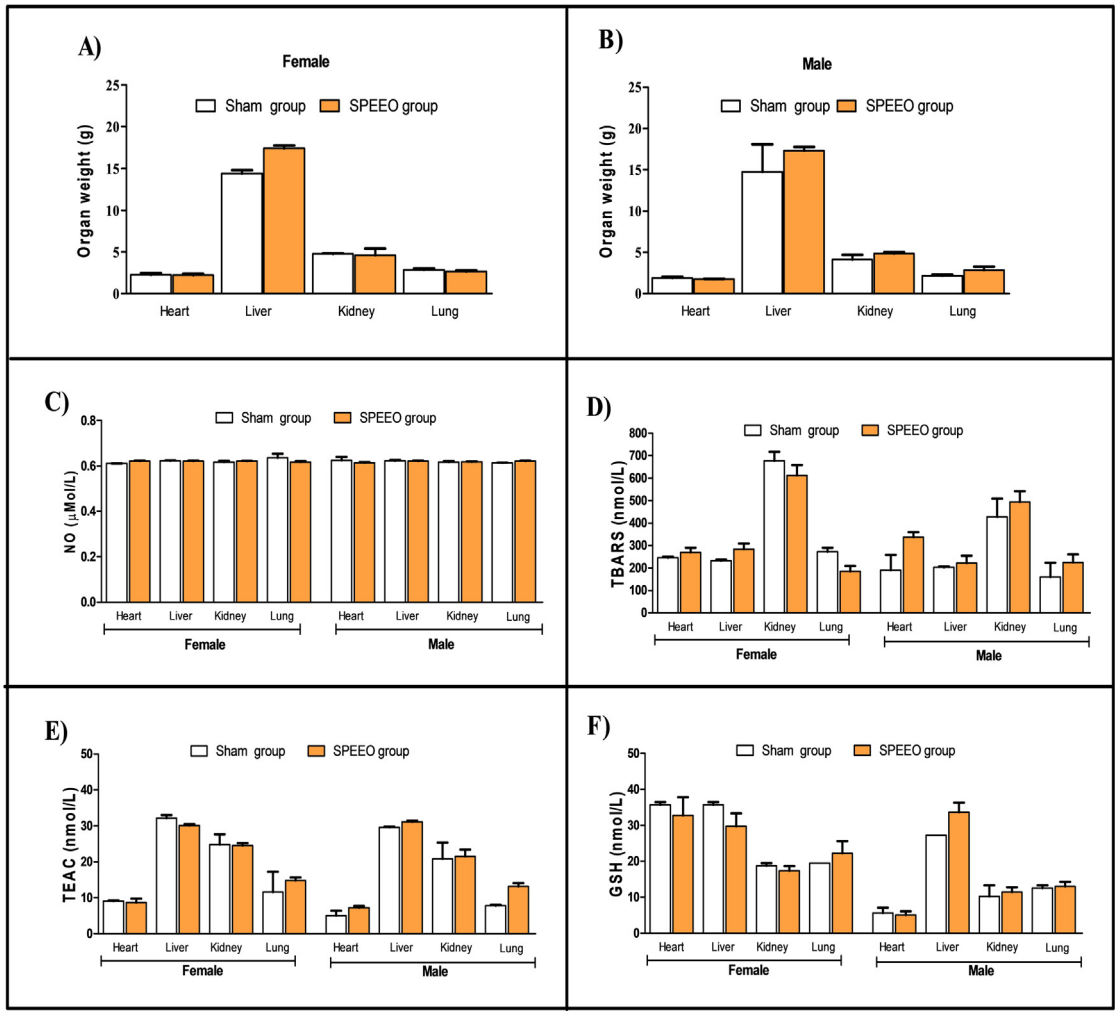

Fig. 4 Organ weight and evaluation of oxidative stress parameters of mice treated with 1000 mg/kg of SPEEO or untreated 14 days after treatment. Note: (A) Organ weight of female group; (B) Organ weight of male group; (C) Nitric oxide production in heart, liver, kidney, and lung of both sexes; (D) TBARS levels in heart, liver, kidney, and lung of both sexes; (E) TEAC levels in heart, liver, kidney, and lung of both sexes; (F) GSH levels in heart, liver, kidney, and lung of both sexes. GSH: glutathione; TBARS: Thiobarbituric acid reactive substances; TEAC: Trolox Equivalent Antioxidant Capacity; SPEEO: *S. pulcherrimum* ethanolic extract ointment.

reported in the literature (Henriques et al., 2016) but their effectivity depends on the type of extraction, the part of the plant, and the species (Grice and Segre, 2011). A approach with hydroalcoholic extract of *Stryphnodendron adstringens* against *S. aureus* reported the antibacterial activity of that extract at high concentrations (200, 400, and 600 µg/mL) (Almeida et al., 2017). However, this represents a problem, due to the possible adverse effects of using the extract from this tree at high concentrations for treating certain affections.

Skin infections may be mainly due to *S. aureus*, *Streptococcus pyogenes*, *E. coli* and *P. aeruginosa* (Bowler et al., 2001; Williams et al., 2017). In addition, the presence of bacteria in skin ulcers can lead to worsening of the wound healing process. In this sense, an ointment with antibacterial action against *S. aureus* promoting wound healing is interesting. Therefore, a non-toxic preparation applied topically to wounds would be a new interesting tool to fight those kind of insults.

In the present study, following the regulation of Brazilian National Health Surveillance Agency (ANVISA) approved in ordinance no. 1.1163/2009 (De Estado Da Saúde et al., 2011) for topical treatments of wounds, we tested the effects of the SPEEO. The first step was to clearly establish a lack of local toxicity in mice, as well as following the main toxicological parameters in those animals, in order to ensure the general safety of the preparation and of the treatment.

Cytotoxicity was tested, as recommended (Parasuraman, 2011) to the better cellular marker than 3T3-A31 (Frión-Herrera et al., 2014), namely, MRC-5 fibroblasts and showed no cytotoxicity.

Interestingly, the administration by gavage of crude extract of *S. adstringens* stem bark (3000–5000 mg/kg), animals presented hypoactivity, hyperventilation, ptosis, hypothermia, motor impairment, sedation, and catatonia, reversible within 48 h while already at 800 mg/kg of this extract showed acute and chronic toxic effects in Wistar rats treated by gavage (Almeida et al., 2017).

In the present experiments of dermal application of up to 1000 mg/kg of SPEEO did not reveal any edema or erythema compared to sham. Furthermore, and obviously, the treatment

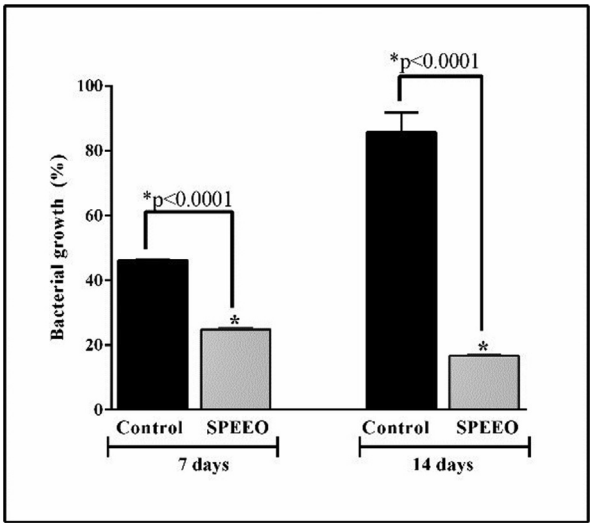

Fig. 5 Percentage of bacterial growth in mice treated with 1000 mg/kg of SPEEO or Kollagenase® ointment (control) at 7th and 14th days. Note: *Significant difference between SPEEO vs control group ($P < 0.001$). SPEEO: S. pulcherrimum ethanolic extract ointment.

did not cause death in any treated mice. During 14 days of treatment, mice temperatures were monitored, and showed no changes. The animals had increased weight and normal food and water consumption, demonstrating their wellness, leading to the conclusion that the preparation was well tolerated. Furthermore, their hematological parameters remained within the reference values. Although male mice had lower platelet count compared to sham, these values are within values normal to male Swiss mice (Reste et al., 2014). Regarding biochemical parameters, an alteration of AST was observed in both sexes, but ALT was within the reference values, translating in the absence of liver damage (Kwo et al., 2017). Finally, the macroscopic postmortem examination of the organs as well as their absence of weight variations (Piao et al., 2013) (heart, lung, liver, and kidney) of the SPEEO treated mice showed no abnormalities. The data of analysis of oxidative stress and antioxidant capacity on the organs of mice treated with SPEEO also showed no alterations in the evaluated parameters. From the toxicological point of view, the topic administration of SPEEO led to no sign of toxicity, strongly suggesting a poor absorption of the SPEEO active components by the skin.

The biological samples taken from the mice's wounds showed the presence of the bacterium of the genus *staphylococcus*. Our study corroborates the study by (Nakatsuji et al., 2018), which identified bacteria of the same gender in the skin of mice. *S. aureus* is part of the skin and mucosa microbiota in humans; however, it can become pathogenic when invading tissues. The *in vivo* antibacterial action results of SPEEO clearly showed inhibition of *Staphylococcus* sp. By comparison, Kollagenase® ointment (used as a control treatment) was significantly less effective against *S. aureus*. The negative control ointment was collagenase combined with chloramphenicol, a broad-spectrum antibiotic that inhibits bacterial protein synthesis (Dinos et al., 2016). This finding may explain the increase of *S. aureus* bacterial load after 14 days of treatment with Kollagenase® (Mandelbaum et al., 2003).

Regarding SPEE, the data showed that this ointment has excellent *in vivo* antibacterial action, which can be due to the compounds present in SPEE, such as gallic acid as reported previously (Liu et al., 2017). The mechanisms by which gallic acid may act is linked to the loss of synthesis and the accumulation of polysaccharide intercellular adhesin (PIA) of the cell surface of *S. aureus* with the deficient formation of *S. aureus* biofilm (Cerca et al., 2008; Liu et al., 2017; Yu et al., 2017).

Furthermore, the SPEE also contains condensed tannins, such as catechin, isomers of Procyanidin-prodelphinidin dimer (B type) and (epi)catechin-(epi)catechin-(epi)gallocatechin (Giffoni de Carvalho et al., 2020; Olšovská et al., 2013). The mechanism of antibacterial action for condensed tannins is based on its capacity of molecular complexation with proteins through nonspecific forces, such as hydrogen bonds and hydrophobic effects, as well as through the formation of covalent bonds (Min et al., 2007; Peng et al., 2018) leading to the inactivation of many cellular transport systems (Gupta and Birdi, 2017; Upadhyay et al., 2014). Also, bacterial inhibition by tannins may be due to the inhibition of enzymes of the fatty acid biosynthesis pathway (FAS II) in bacteria (Trevisan et al., 2020).

5. Conclusions

The presented data, for the first time, prove the excellent antibacterial action of the *S. pulcherrimum* extract ethanolic based ointment. We were able to putatively identify thirteen compounds, including simple organic acid derivatives and condensed tannins oligomers. The *in vivo* tests with SPEEO showed that the ointment-based extract of this species had good absorption and did not cause irritation to the skin of the mice at a dose of 1000 mg/kg, suggesting a wide safety margin at therapeutic doses. The elevated *in vivo* and *in vitro* bacterial inhibitions obtained with the SPEE are important findings in this study because this extract can be used as a potential and new therapeutic alternative against skin infection by *S. aureus*, which is a highly resistant bacteria. The tannins found in the SPEE can be the origin of the antibacterial and cytotoxic actions because those components can complex cellular enzymes inhibiting their activity. It is possible, though, that these activities may also be due to the synergistic action between tannins and other components found in the extract. Therefore, future studies will be needed to determine the mechanism of action or whether other chemical compounds in addition to the tannins present in the extract also contributed to these properties.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103183.

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