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Biological activity of geopropolis produced by *Partamona cupira* (Meliponinae, Apidae) in the semiarid of the Brazilian northeast

Atividade biológica da geoprópolis produzida por *Partamona cupira* (Meliponinae, Apidae) no semiárido do nordeste brasileiro

Actividad biológica del geopropólis producido por *Partamona cupira* (Meliponinae, Apidae) en la región semiárida del noreste de Brasil

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Jael Soares Batista
ORCID: https://orcid.org/0000-0001-9663-7468
Federal Rural Semi-Arid University, Brazil
E-mail: Jaelsoares@hotmail.com
Jardel Bezerra da Silva
ORCID: https://orcid.org/0000-0001-6462-3328
Federal Rural Semi-Arid University, Brazil
E-mail: jardelbezerra@bol.com.br
Kizzy Millenn de Freitas Mendonça Costa
ORCID: https://orcid.org/0000-0001-9234-9485
University of the State of Rio Grande do Norte, Brazil
E-mail: kizzymillenn@gmail.com
Tiago da Silva Teófilo
ORCID: https://orcid.org/0000-0002-3021-0880
Federal Rural Semi-Arid University, Brazil
E-mail: tiago.teofilo@ufersa.edu.br
Natanael Silva Félix
ORCID: https://orcid.org/0000-0002-9631-2847
Federal Rural Semi-Arid University, Brazil
E-mail: natann-013@hotmail.com
Francisco Herbeson Aquino Silva
ORCID: https://orcid.org/0000-0002-9653-5442
Federal Rural Semi-Arid University, Brazil
E-mail: herbeson2706@hotmail.com

Luciana Cristina Borges Fernandes
ORCID: https://orcid.org/0000-0001-6920-2645
Federal Rural Semi-Arid University, Brazil
E-mail: lucianacbf@hotmail.com
Wanderson Lucas Alves Dos Santos
ORCID: https://orcid.org/0000-0002-6583-0481
Federal Rural Semi-Arid University, Brazil
E-mail: wandersonlucas93@hotmail.com
Wesley Lyeverton Correia Ribeiro
ORCID: https://orcid.org/0000-0002-2784-0013
Federal University of Ceará, Brazil
E-mail: wesleylyeverton@yahoo.com.br
Geysa Almeida Viana
ORCID: https://orcid.org/0000-0002-3339-446X
Centro Universitário UNINTA, Brazil
E-mail: geysaalmeidav@hotmail.com

Abstract

Research on the chemical composition and pharmacological activities of geopropolis produced by stingless bees (Hymenoptera, Apidae, Meliponini) may contribute to expand its use of propolis-based formulations in the clinical context. Thus he study aimed to evaluate the chemical composition and biological activity of the hydroethanolic extract (HEG) of the geopropolis of *Partamona cupira*, obtained in the semiarid region of northeast Brazil. Chemical analyses of HEG were carried out using HPLC-DADESI-MS/MS. The antioxidant activity of extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay method and cytotoxic activity by the *in vitro* MTT method [brometo de 3- (4.5dimetiltiazol-2-il)-2.5-difeniltetrazolio]. The antibacterial activity of the HEG was evaluated through the disc-diffusion test on agar and measurement of the promoted by the extract in different concentrations. The genoprotective potential of the HEG was evaluated through the comet assay on fibroblasts of L929, co-treated with the extract and submitted to genotoxicity induction with H_2O_2. We also investigated the healing effect of the cream containing geopropolis (10%) on experimental skin wounds in Wistar rats. The HEG presented in its composition phenolic compounds of high biological activity, as well as revealed high antioxidant activity and promoted genoprotective effect by reducing DNA damage from L929

fibroblasts. The HEG presented antimicrobial activity promoting inhibition of *S. aureus*, *S. pyogenes*, *E. coli* and *E. aerogenes*. The topical use of the cream containing geopropolis promoted wound closure and faster reepithelialization in relation to the control group, in addition to a less intense inflammatory reaction, increased fibroblastic proliferation and collagen deposition.

Keywords: Meliponinae; Geopropolis; Chemical composition; Antioxidant activity; Genoprotective potential; Healing effect.

Resumo

As pesquisas sobre a composição química e as atividades farmacológicas de geoprópolis produzidas por abelhas sem ferrão (Hymenoptera, Apidae, Meliponini) podem contribuir para ampliar o uso de formulações à base de própolis no contexto clínico. Assim, o estudo teve como objetivo avaliar a composição química e a atividade biológica do extrato hidroetanólico (HEG) da geoprópole de *Partamona cupira*, obtido na região semiárida do nordeste brasileiro. As análises químicas de HEG foram realizadas usando HPLC-DADESI-MS / MS. A atividade antioxidante dos extratos foi avaliada pelo método do ensaio 2,2-difenil-1-picrilhidrazil (DPPH) e a atividade citotóxica pelo método MTT *in vitro* [brometo de 3-(4,5dimetiltiazol-2-ilo) -2,5-difeniltetrazólio]. A atividade antibacteriana do HEG foi avaliada através do teste de disco-difusão em ágar e dosagem, promovido pelo extrato em diferentes concentrações. O potencial genoprotetor do HEG foi avaliado através do ensaio do cometa em fibroblastos de L929, co-tratados com o extrato e submetidos à indução de genotoxicidade com H_2O_2. Também investigamos o efeito cicatrizante do creme contendo geoprópolis (10%) em feridas cutâneas experimentais em ratos Wistar. O HEG apresentou em sua composição compostos fenólicos de alta atividade biológica, bem como revelou alta atividade antioxidante e promoveu efeito genoprotetor ao reduzir os danos ao DNA dos fibroblastos L929. O HEG apresentou atividade antimicrobiana promovendo inibição de *S. aureus*, *S. pyogenes*, *E. coli* e *E. aerogenes*. O uso tópico do creme contendo geoprópolis promoveu fechamento da ferida e reepitelização mais rápida em relação ao grupo controle, além de reação inflamatória menos intensa, aumento da proliferação fibroblástica e deposição de colágeno.

Palavras-chave: Meliponinae; Geoprópolis; Composição química; Atividade antioxidante; Potencial genoprotetor; Atividade cicatrizante.

Resumen

La investigación sobre la composición química y las actividades farmacológicas de los geopropólis producidos por abejas sin aguijón (Hymenoptera, Apidae, Meliponini) pueden contribuir a expandir su uso de formulaciones a base de propólis en el contexto clínico. Así, el estudio tuvo como objetivo evaluar la composición química y la actividad biológica del extracto hidroetanólico (HEG) del geopropólis de *Partamona cupira*, obtenido en la región semiárida del noreste de Brasil. Los análisis químicos de HEG se llevaron a cabo usando HPLC-DADESI-MS / MS. La actividad antioxidante de los extractos se evaluó usando el método de ensayo 2,2-difenil-1-picrilhidrazilo (DPPH) y la actividad citotóxica por el método MTT *in vitro* [bromuro de 3- (4,5 dimetiltiazol-2-ilo) -2,5-difeniltetrazolio]. La actividad antibacteriana del HEG se evaluó mediante la prueba de difusión en disco en agar y la medición del promovido por el extracto en diferentes concentraciones. El potencial genoprotector del HEG se evaluó mediante el ensayo cometa en fibroblastos de L929, se co-trató con el extracto y se sometió a inducción de genotoxicidad con H_2O_2. También investigamos el efecto curativo de la crema que contiene geopropóleo (10%) en heridas experimentales de la piel en ratas Wistar. El HEG presentó en su composición compuestos fenólicos de alta actividad biológica, además de revelar una alta actividad antioxidante y promover el efecto genoprotector al reducir el daño al ADN de los fibroblastos L929. El HEG presentó actividad antimicrobiana promoviendo la inhibición de *S. aureus*, *S. pyogenes*, *E. coli* y *E. aerogenes*. El uso tópico de la crema que contiene geopropóleo promovió el cierre de la herida y una reepitelización más rápida en relación al grupo control, además de una reacción inflamatoria menos intensa, aumento de la proliferación fibroblástica y deposición de colágeno.

Palabras clave: Meliponinae; Geopropolis; Composición química; Actividad antioxidante; Potencial genoprotector; Efecto curativo.

1. Introduction

The bees of the subfamily Meliponinae (Hymenoptera, Apidae) present a wide geographical distribution in Brazil, with more than 250 species, distributed in 27 genera (Silveira et al., 2002). In Brazil, stingless bees have been raised for centuries by traditional populations, and this activity is called meliponiculture. Meliponiculture is considered a sustainable activity that helps to preserve bees and the environment, through the pollination service provided to native plants, besides increasing the income of family farmers (Lima-

Verde et al., 2019). Meliponiculturists capture in nature hive species of bees adapted to local vegetation and climate conditions such as: *M. subnitida, P. mosquito, M. asilvae, M. scutellaris, P. cupira, Frieseomelitta spp e F. varia* and transfer them to wooden boxes for the purpose of making the hives more productive and makes the collection of honey and propolis but easy, fast and hygienic (Silva et al., 2016).

Among the bees belonging to the subfamily Meliponinae, *Partamona cupira* stands out, which frequently occurs in areas of dry vegetation of the caatinga biome of Northeast Brazil (Silveira et al., 2002). They are docile bees that build their hives in pre-existing cavities, which are difficult to access and make it difficult for predators to enter, such as termite mounds or openings in trees (Hrncir et al., 2017). In a study to discover the species of stingless bees raised in hives in the state of Rio Grande do Norte (Northeast, Brazil), Pereira et al. (2011) found that *Partamona cupira* was present in 4.8% of the hives.

In popular medicine, several medicinal properties are attributed to propolis and geopropolis produced by meliponids (Silva et al., 2016). After investigation of the biological activity propolis and geopropolis of Brazilian stingless bee Lavinas et al. (2019) found promising therapeutic properties and absence of toxicity of its extracts, being its use considered safe**.** Aiming to seek new knowledge about the quality of products produced by bee species belonging to the subfamily Meliponinae was structured and this study aimed to assess the chemical composition and biological activity the hydroethanolic extract of the geopropolis of the stingless bee *Partamona cupira* obtained in the semiarid do northeast do Brazil.

2. Material and Methods

2.1 Obtaining geopropolis samples and preparing extracts

The taxonomic classification of bees was carried out at the Zoology Laboratory of the Federal Rural University of Semi-Arid (Mossoró, RN, Brazil), as described by Camargo (1980). The samples of geopropolis were collected in April 2018 from two apiaries in the city of Mossoró, Rio Grande do Norte, Brazil (5º11'15″ S, 37º20'39″ W). The region has a tropical semiarid hot climate with an average annual temperature of 27°C, relative temperature of 50%, and annual average precipitation of 500 mm.

To obtain the extract, 40 g of raw geopropolis was crushed in a Walita 500 W blender and transferred to a flat bottom balloon. Ethyl alcohol (70%) was added at a ratio of 5:1;

approximately 5 mL of alcohol was used for every gram of crushed geopropolis and the mixture was kept at room temperature. Next, the supernatant was filtered, and the extract was subjected to rotoevaporation at 40°C until it completely dried and ethanol was removed. After solvent evaporation, the dry extract was obtained, the material was weighed, and the yield was calculated in relation to the initial mass used and expressed as a percentage.

2.2 Chemical composition analysis

The sample constituents of geoproplis hydroalcolic extract (HEG) were characterized by the HPLC-DAD-ESI-MS / MS technique. The equipment used was a DADSPD-M10AVP chromatograph (Shimadzu Corp., Kyoto, Japan), the chromatograph was coupled to a Esquire 3000 Plus mass spectrometer (Bruker Daltonics, Billerica, MA, USA), equipped with a quadruple ion trap mass analyzer, according to the methodology described by Ferreira et al. (2017). All compounds were characterized by the interpretation of the respective ultraviolet (UV) and mass spectra, MS literature data and Scifinder® online chemical databases (http://www.scifinder.org), Reaxys® (http: //www.reaxys.com), Riken MSn Spectral database for phytochemicals (Respect) (http://www.reaxys.riken.jp), Phenol - Explorer (http://phenol-explorer.eu), Chem.Spider (http: // www.chemspider.com) and HMDB (http://www.hmdb.ca).

2.3 Antioxidant activity

The antioxidant capacity of the extract was tested by the *in vitro* phototocolorimetric method of the free radical DPPH (2.2-diphenyl-1-picrylhidrazil) described by Mensor et al. (2001). The sequestrating capacity of the DPPH radical was represented by the IC50 values (%) of the samples compared to quercetin, used as reference standard.

2.4 Evaluation of cytotoxic activity

The cytotoxic activity of HEG was evaluated by colorimetric assay using the methyl thiazole tetrazol reagent (MTT). The assay was performed in accordance with the standards of the International Organization Standardization (ISO) number 10993-5: 2009. The extract was tested on L929 fibroblasts, sown in 96-well culture plates, exposed for 24 hours to different extract concentrations (500, 250, 125, 62.5, 31.2 and 15.6 $\mu g.mL^{-1}$). After the exposure period, 1 mL of MTT solution (0.5 $mg.mL^{-1}$) was added to each well. Dimethylsulfoxide

(DMSO) was used as a negative control. To quantify the conversion of MTT (yellow stain) to formazan (purple stain) by living cells, colorimetric quantification was performed with the aid of a spectrophotometer, at a wavelength of 570nm, with the absorption value being proportional to the number of viable cells (cell viability). The mean absorbance data obtained from the test solution and negative control were compared by analysis of variance ANOVA, followed by Tukey's post-test using the GraphPad Prism software version 5.0 (GraphPad Software, San Diego, CA, USA), with a significance level of $P<0.05$.

2.5 Evaluation of genoprotective potential

The genoprotective effect of HEG was evaluated by the comet assay on fibroblasts of the L929 line (0.7x105cells/mL), cultivated in DMEM (Dulbecco Modification of Minimum Essential Media; GIBCO®), supplemented with 10% bovine fetal serum and 1% antibiotics. L929 cells were exposed to hydrogen peroxide (H_2O_2; 150 µm per 2h of exposure) for genotoxicity induction (positive control) and sterile distilled water (negative control). Cell cultures were also co-treated with increasing concentrations of geopranol samples (100, 250 and 500 $\mu g.mL^{-1}$) and H_2O_2 (150 µm) for 2h at 37°C in a 5% CO_2 atmosphere (test group).

The degree of DNA lesion was identified visually through the analysis of the tail formed by the fragments of DNA, being that the size of the tail was proportional to the dimension of the damage caused, indicating the degree of lesion suffered by the cell (Lovell et al., 1999). The protection effect of the test samples on the genotoxicity induced by H_2O_2 was calculated according to Waters et al. (1990), according to the formula: % Reduction = (A-B/A-C) x 100. Where A corresponds to the ID induced by H_2O_2, B corresponds to the ID induced by the anti-genotoxic treatment (H_2O_2 + test sample) and C corresponds to the ID assigned to the negative control (distilled water).

2.6 Evaluation of antibacterial activity

The antibacterial activity of the HEG sample was evaluated by the disc-diffusion agar test associated with the well drilling technique (Ostrosky et al., 2008). The bacterial strains used came from standardized collections American Type Cell Culture (ATCC), duly morphologically, physiologically and biochemically characterized, being the assays performed with Gram-positive bacteria *S. aureus* (ATCC 6538), *S. pyogenes* (ATCC 19615) and Gram-negative *E. coli* (ATCC 25922) and *E. aerogenes* (ATCC 1304). The analysis of

the sensitivity of bacteria against HEG determined at concentrations of 100, 50 and 25, 12.5, 6.25 mg.mL^{-1}. The test was accompanied by positive controls the antibiotic doxycycline in the concentration of 20 µg and as negative control distilled water was used.

The results were obtained by measuring the diameter of the inhibition halos around the wells containing different concentrations of the tested solution. The measurement was performed with the aid of a pachymeter and the results were expressed in millimeters, being considered as positive antimicrobial activity the halos of size less than or equal to 9 mm, as described by Carvalho et al. (2014). Differences between the inhibition halos promoted by the positive controls and the extract concentrations were verified by analysis of variance, followed by Tukey's post-test at 5% significance ($p < 0.05$).

2.7 *In vivo* healing activity

The effects of the topical treatment of the cream based on geopropolis were evaluated using 32 rats (*Rattus norvegicus* Berkenhout, 1769), Wistar lineage, males, 60 days old, with body weight of 250g. These animals were randomly distributed into two experimental groups. Group I consisted of 16 rats with skin wounds, treated with topical application of the cream containing 10% geoprolis; and group II, control, with the same number of animals, but with topical application of Lanette® base cream without the addition of geopropolis.

To perform the excisional skin lesions, the animals were anesthetized with dissociative anesthesia using xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (140 mg/kg) administered intramuscularly. After anesthesia, each animal was submitted to trichotomy and induction of a circular lesion of 1 x 1 cm in diameter in the skin of the back lumbar region using a scalpel blade, scissors and dissection forceps. The cream was applied twice a day for 21 days with the aid of sterile, individual wood spatulas, forming a film that covered the entire surface of the experimental wound.

In all animals the skin lesions were observed daily for the presence of hyperemia, edema, bleeding, secretion, odor, and crust formation. These symptoms were scored as follows: 0 (absent), 1 (mild), 2 (moderate) and 3 (accented).

To evaluate the effect of the extract on the evolution of the cutaneous lesions closure, the area of the lesions on the 3rd, 7th, 14th and 21st after surgery was measured using a pachymeter. The closure of the lesions was then evaluated by the degree of contraction, expressed as a percentage, which was measured by the equation proposed by Ramsey et al. (1995), where Wo = initial area of the wound and Wi = area of the wound on the day of

biopsy:100 x (Wo - Wi)/ Wo = % contraction. For statistical analysis of the results, the non-parametric Kruskal-Wallis test and comparisons of means using the Student's T test were used.

The wounds were biopsied using dermatological punch for skin biopsy on the 21 day after surgery. The fragments obtained from the biopsies were fixed in 10% formalin and submitted to paraffin inclusion to obtain 5μm thick sections, stained with hematoxin and eosin. A descriptive histological analysis of skin changes was then performed and the histological elements, which indicate the evolution of the healing process, were evaluated:

polymorphonuclear cells, mononuclear cells, blood vessels, fibroblasts and collagen fibers. These histological parameters were grouped as absent, mild, moderate or intense, following the criteria described by Akkol et al. (2008). The occurrence of epidermal layer restoration (reepithelialization) was verified in each histological slide, describing whether it was absent, partial, or total.

The experimental protocols used in this study are in accordance with the precepts of Law 11.794 of October 8, 2009 and the standards issued by the National Council for Animal Experimentation (CONCEA), they were approved by the Ethics Committee for Animal Use (CEUA) of the State University of Rio Grande do Norte (UERN), with protocol number 006/15.

3. Results

3.1 Chemical composition

The evaluation using the HPLC-DAD-ESI-MS / MS technique demonstrated the HEG had a varied chemical composition, being detected 20 phenolic compounds, distributed between flavonoids and phenol. In the group of flavonoids it was possible to identify chalcones (2', 6',4'-trihydroxy-4-methoxy chalcone, 2',6',4-trihydroxy chalcone, 2',6',4-trihydroxy-3,4'-dimethoxy chalcone, 4'-methoxy-2',6',4- trihydroxy chalcone, 6'-methoxy-2',4-dihydroxy chalcone, 4'-methoxy-2',6',4- trihydroxy chalcone, 6'-methoxy-2',4-dihydroxy chalcone), flavones (luteolin methyl éter, tricin – trihydroxy dimethoxy flavone, trihydroxy trimethoxy flavone, tricin methyl ether, luteolin dimethyl ether, ethoxybenzyl alcohol acetyl glucoside) and flavonols (kaempferol, rhamnetin, kaempferol dimethyl ether, isorhamnetin, quercetin). The group of phenols were identified (sinapoyl phenyl ester and 5-Hexadecyl cardanol). The analyses also showed differences in the relative abundance of the

extract constituents, with the compounds 6'-methoxy-2',4-dihydroxy chalcone, tricin - trihydroxy dimethoxy flavone, tricin methyl ether, quercetin expressing the highest peak at retention time (Table 1).

Table 1. Constituents of the geopropolis extract *Partamona cupira* (Meliponinae, Apidae) from the semiarid region of Northeast Brazil analyzed by HPLC-DAD-ESI-MS / MS.

Flavonoids							
Chalcones	TR (min.)	Flavones	TR (min)	Flavonols	TR (min.)	Phenols	TR (min.)
2', 6',4'-trihydroxy-4-methoxy chalcone	31,441	Luteolin Methyl éter	21,9	Kaempferol	26,4	Sinapoyl phenyl ester	13,9
2',6',4-trihydroxy chalcone	31,441	Tricin – Trihydroxy dimethoxy flavone	41,3	Rhamnetin	26,9	5-Hexadecyl cardanol	15,430
2',6',4-trihydroxy-3,4'-dimethoxy chalcone	33,123	Trihydroxy trimethoxy flavone	23,9	Kaempferol dimethyl ether	26,4	__	__
4'-methoxy-2',6',4-trihydroxy chalcone	31,441	Tricin methyl ether	41,3	Isorhamnetin	27,244	__	__
6'-methoxy-2',4-dihydroxy chalcone	41,3	luteolin dimethyl ether	21,9	Quercetin	33,129	__	__
4'-methoxy-2',6',4-trihydroxy chalcone	33,123	4-Ethoxybenzyl alcohol acetyl glucoside	24,8	__	__	__	__
6'-methoxy-2',4-dihydroxy chalcone	41,3	__	__	__	__	__	__

TR: retention time. Source: Authors.

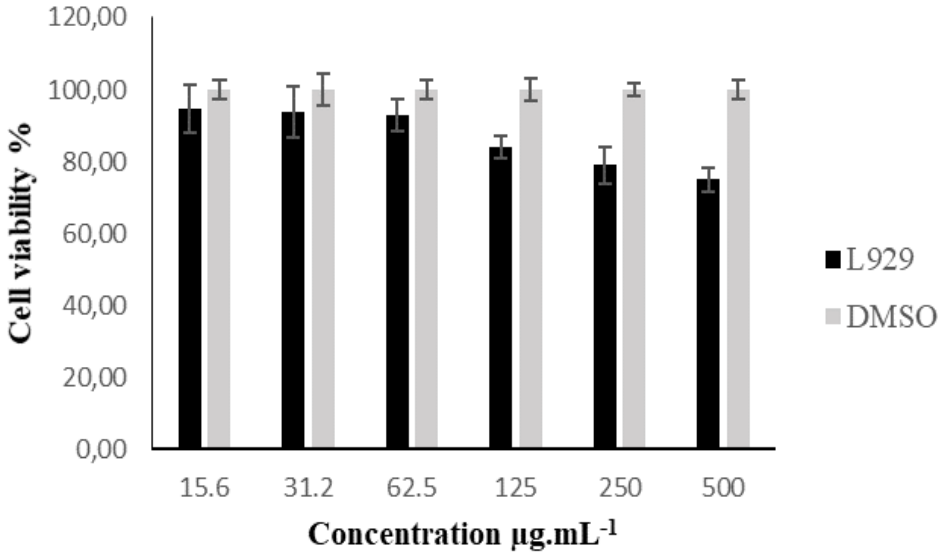
3.2 Antioxidant activity

The results obtained in the DPPH free radical sequestration capacity test showed that the inhibitory concentration required to decrease the free radical DPPH (IC50) by 50% was 48 $\mu g.mL^{-1}$.

3.3 Cytotoxic activity

There has been a gradual increase in cell viability as extract concentration has been reduced. Thus, from the dilution of 125 µg.mL^{-1}, there was no significant difference in the viability percentages relative to the negative control (DMSO) (Figure 1).

Figure 1. Viability of fibroblasts (L929) analyzed by colorimetric test using MTT after 24 hours of exposure to different concentrations of *Partamona cupira* hydroalcoholic extract.



Source: Authors.

3.4 Evaluation of genoprotective potential *in vitro*

In all tested concentrations, HEG promoted reduction of genotoxicity induced by H_2O_2 in L929 fibroblasts. The percentages of reduction of genotoxicity were 92.2%, 74.5% and 46.1%, respectively, for the concentrations of 500, 250 and 100 µL/mL (Table 2)

Table 2. Mean ± standard deviation and percentage of genotoxicity reduction, analyzed by comet assay, to evaluate the genoprotective effect of *Partamona cupira* geopropolis extract, in cells of L929 lineage, co-treated with three concentrations of propolis and hydrogen peroxide samples.

Parameter analyzed	Extract concentration (µL/mL)		
	500	250	100
% reduction of genotoxicity	92,22 ± 3,38	74,57 ± 2,95	46,10 ± 5,36

Source: Authors.

3.5 Antibacterial activity

In vitro antibacterial activity assays showed that HEG was able to promote inhibition halos for all tested bacteria. As the concentration of the extract increased, there was also enlargement of the inhibition halos. At concentrations of 100 mg.mL^{-1}, 50 mg.mL^{-1} and 25 mg.mL^{-1}, all tested bacteria showed inhibition halos with size ≥ to 9 mm. The results obtained in this study also showed that the concentrations of 100 mg.mL^{-1} provided excellent results, since the averages of the inhibition halos promoted for the bacteria *S. aureus*, *S. pyogenes* and *E. coli* did not differ significantly ($P > 0.05$) from the halos promoted by the control antibiotic. Regarding the size of the inhibition halo, it can be observed that *S. aureus* showed a higher sensitivity to HEG (Table 3).

Table 3. Mean and standard deviation of halos sizes of inhibition (mm) of bacterial growth promoted by *Partamona cupira* geopropolis extract in different dilutions and antibiotic doxycycline (control +), analyzed through the Agar diffusion method.

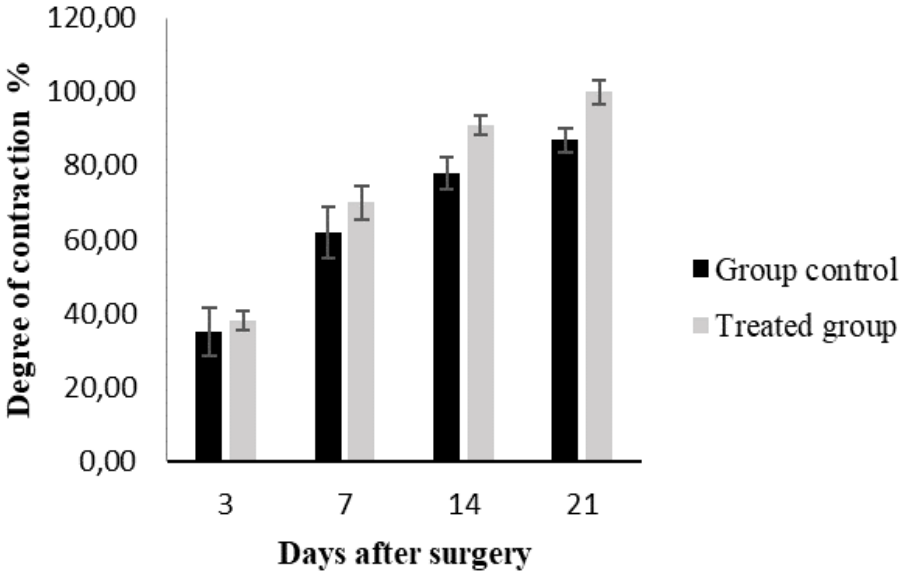
	Extract concentration (mg.mL^{-1})					
Bacteria	100	50	25	12.5	6.25	Contr. + Antibiot.
S. aureus	*16.0±4,0a	15.0±3,6a	11±5,6b	8.3±6,4bc	7,3±4,0c	17.0±4,8a
S. pyogenes	14±7,6a	10.0±6,1b	9.0± 3,6b	8.0±6,5b	7.0±4,9 b	16.0±4,8a
E. coli	13.0±4,0a	10,0±4,9b	10.0± 5,2b	8.0±4,3b	7.3±4,0b	15.0±3,7a
E. aerogenes	12.0±3,2b	11.0±4,3b	11.0±3,5b	8.0±4,4b	7,0±3,6b	19.0±6,0a

* Values referring to the size mm of the inhibition halos.
Different letters on the lines indicate statistical difference by Tukey test ($p < 0.05$).
Source: Authors.

3.6 *In vivo* healing activity

Applying the formula established for the calculation of the average percentage of contraction, it was possible to observe that the skin lesions of G1 animals presented respectively 38.0, 70, 91.0 and 100% of the initial area reduction on days 7, 14 and 21, while in G2 animals the reduction of the lesion area in the same period was of 35.0, 62.0, 69.0, 78%, being therefore verified significant difference between the groups from the 14th after the surgery (Figure 2).

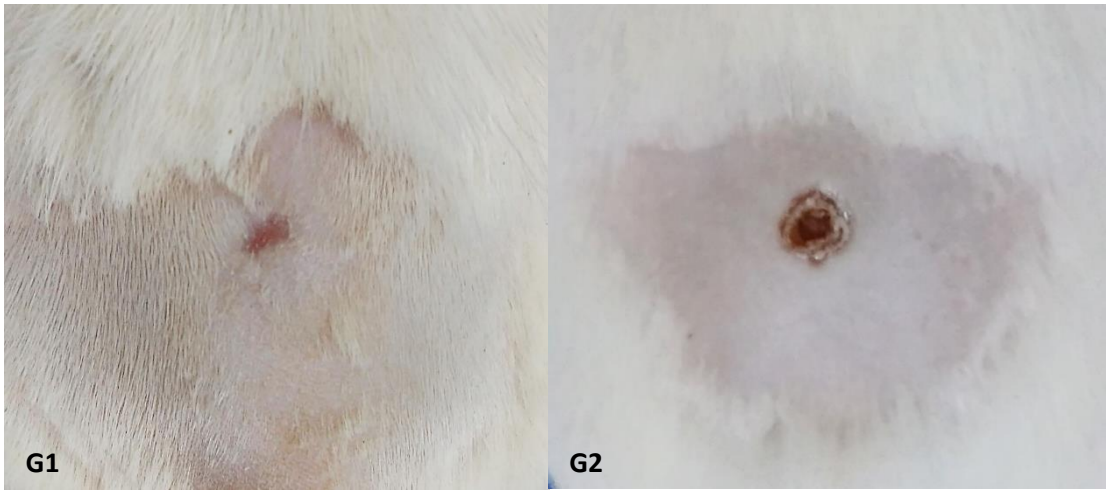
Figure 2. Average value of the percentage of contraction (%) of the wound areas at the 3rd, 7th, 14st and 21st day of evolution after surgery of rats treated with cream containing *Partamona cupira* geopropolis and control group.



Source: Authors.

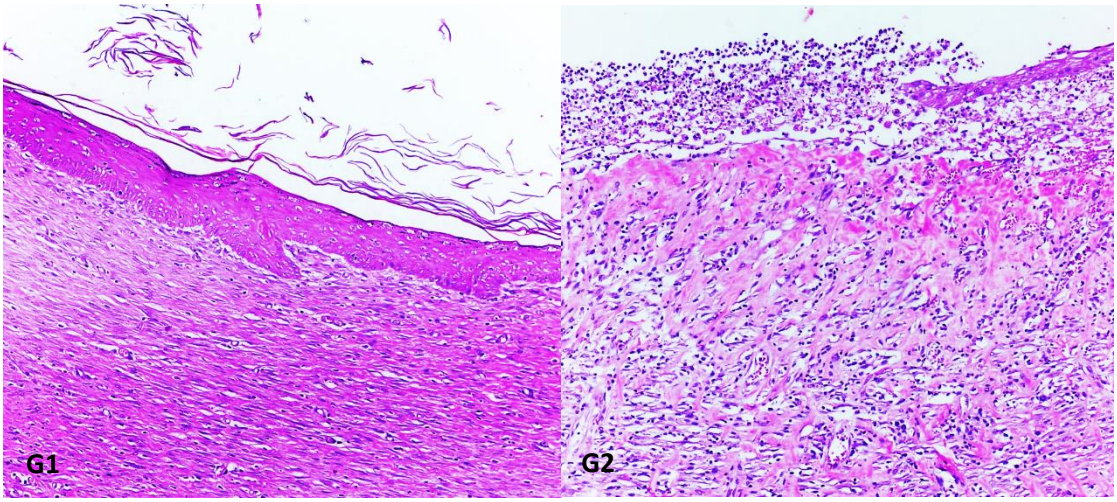
The macroscopic evaluation of the skin lesions revealed notable differences between the groups. In G1, they presented lesions with a clean, dry and completely closed appearance at the end of the experimental period. While the lesions of the animals belonging to G2 presented swollen, humid, with presence of inflammatory exudate, covered by crust and not totally closed (Figure 3 G1 and G2). In the microscopic analysis of the lesions collected 21 days after surgery it was observed complete wound healing, evidenced by intense density of fibroblasts and collagen fibers, presence of a few vessels spacedly isolated, besides the complete regeneration of the epidermis. In the same period, the histological findings of the G2 animals were the presence of granulation tissue which was evidenced by the presence of a moderate amount of blood capillaries, mononuclear inflammatory cells, fibroblasts and collagen fibers. In the animals of this group, the regeneration of the epidermis was partial (Figure 4 G1 and G2).

Figure 3. Macroscopic aspects of skin wounds in rats treated with cream containing geopropolis (G1) and control group (G2) on the 21st day after surgery. Note in G1 the completely healed wound. G2 wound not completely closed and covered with crust.



Source: Authors.

Figure 4. Histological aspects of skin wounds in rats treated with cream containing geopropolis (G1) and control group (G2) on the 21st day after surgery. H&E stain. 20x. It is noted in G1 completely regenerated epidermis and dermis with presence of collagen and fibroblasts and in G2 partial regeneration of the epidermis and presence of inflammatory cells and granulation tissue in the dermis.



Source: Authors.

4. Discussion

4.1 Chemical composition

Due to the need to obtain more information about the geopropolis composition of the bee *Partamona cupira* the ethanolic extract was evaluated using the HPLC-DAD-ESI-MS technique in order to identify the classes of compounds present in it. Thus, this study showed that the extract had a varied chemical composition, being detected 20 different types of phenolic compounds. Data on the chemical composition of the *Partamona cupira* bee geopropolis are still scarce in the literature. Chemical studies of propolis from other stingless bees, such as *Melipona scutellaris* and *Plebeia aff flavocincta* (Franchin et al., 2012), both studies also revealed high levels of phenolic compounds. The chemical profile obtained in this paper, characterized by the abundance of flavonols, such as quercetin methyl ethers, and methoxychalcones resembles the distribution of constituents reported in the geopropolis produced by stingless bees of tribe *Meliponini Scaptotrigona postica* in the state of Rio Grande do Norte (RN, northeast Brazil) reported by Ferreira et al. (2017).

4.2 Antioxidant activity

The biological properties of propolis are related to the presence of a variety of biologically active compounds. Due to its high content of phenolic compounds, propolis can present a natural antioxidant effect. Phenolic compounds are a group of non-enzymatic antioxidants that cooperate with the reduction of cellular oxidative damage caused by reactive oxygen species (Nascimento et al., 2010). In this work, we evaluate the antioxidant activity of HEG through the test that evaluates the capacity of the constituents present in geopropolis to promote the free radical reduction of DPPH. Thus, the lower the value of IC50, the greater is the antioxidant capacity, since a smaller amount of the substance will be necessary to promote a 50% reduction in the reagent. According to Reynertson et al. (2005), IC50 below 50 $\mu g.mL^{-1}$ is considered very active. Thus, HEG appeared to be an excellent sequestrating capacity of the DPPH radical, since the inhibitory concentration necessary to decrease the free DPPH radical (IC50) by 50% was 48 $\mu g.mL^{-1}$.

4.3 Cytotoxic activity

One of the most used parameters to evaluate the toxicity of a compound is through cell viability, which can be evidenced by the culture of cells exposed to vital dyes and the test substance (Rogero et al., 2003). The present study was performed using fibroblasts of L929 lineage. Fibroblasts are cells involved in the repair process, are the most common subcutaneous connective tissue cells, whose function is to synthesize extracellular matrix components such as: collagen fibers, reticular fibers and elastic fibers, in addition to producing growth factors that control proliferation and differentiation (Avery et al., 2018).

The metabolic activity of L929 fibroblasts incubated with different concentrations of HEG was submitted to cytotoxicity evaluation by MTT test. The basis of the MTT assay is the interference induced by a certain substance test on cell metabolism and ability of viable cell mitochondria to metabolize tetrazolium salts (Mosmann, 1983). According to ISO (2009), a decline in the number of living cells results in a decline in metabolic activity of the sample. This decline is directly proportional to the amount of blue-violet formazan formed, monitored by the optical density (OD) at 570 nm in the spectrophotometer. Thus, the reduction in the number of viable cells indicates, reduction in mitochondrial function and occurrence of cell death, and high toxicity of a test substance. It can be observed that from the concentration of 250 $\mu g.mL^{-1}$ the cellular viability presented values above 70%. Thus, we found that HEG showed no cytotoxic effect in relation to the cutoff value of 70% defined by the International Organization for Standardization. The absence of cytotoxicity was verified by Lavinas et al. (2019), who found that after investigation of the biological activity propolis and geopropolis of Brazilian stingless bee promising therapeutic properties and absence of toxicity, and their extracts are considered safe in relation to the toxic potential.

4.4 Evaluation of genoprotective potential *in vitro*

Considering the property of forming free radicals of Hydrogen Peroxide and the ability to promote DNA molecule fragmentation, fibroblasts of L929 lineage were co-treated with increasing concentrations of geopropolis samples and submitted to genotoxicity induction with H_2O_2 and analyzed by comet assay. Thus, it was possible to verify that HEG had an antigenotoxic or genoprotective effect since there was a reduction in the percentage of genotoxicity induced by H_2O_2. The literature points out the genoprotective effect of propolis due to the presence in its composition of bioactive compounds, such as phenolic compounds,

which have the capacity to block or at least reduce the action of free radicals (Silva et al., 2016). In this study, the genoprotective effect of red propolis produced by *Apis mellifera* in the state of Rio Grande do Norte (RN, northeast Brazil) was investigated and it was discovered that propolis promoted a significant reduction of DNA damage. Thus, it is possible to suppose that the bioactive substances with antioxidant capacity present in the extract of the bee *Partamona cupira* interacted with the hydrogen peroxide, blocking its genotoxic effect, thus protecting the DNA.

4.5 Antimicrobial activity

The irrational use of synthetic antibiotics has contributed to the emergence of resistant pathogenic microorganisms, highlighting the need for studies to discover new natural substances with antibiotic capacity (Guimarães et al., 2010). The investigation of the antimicrobial activity of bee products produced by stingless bees (Hymenoptera, Apidae, Meliponini), especially propolis and geopropolis, has shown promising results. The ethanol extract of propolis from the bee *Melipona orbignyi* was active against the bacterium *S. aureus* and the fungus *Candida albicans* (Campos, 2014). The hydroalcoholic extract of propolis from *Plebeia sp.* inhibited the growth of *E. aerogenes* and *S. aureus* (Campêlo et al., 2015). The hydroalcoholic extract from geopropolis produced by *Plebeia aff. Flavocincta* inhibited the growth of *S. epidermidis* and *E. coli* (Silva et al., 2016), while ethanolic extracts of propolis from *Melipona quadrifasciata* caused lysis of the bacterial wall and release of intracellular components of *E. coli* and *S. aureus* (Torres, et al., 2018).

In this study, HEG was tested by the Mueller Hinton agar well method against two Gram-positive and two Gram-negative bacteria. We found that HEG was considered effective from the point of view of antimicrobial activity, since it was possible to verify that the extract promoted halo of inhibition ≥9 mm against all the strains tested. Another important data refers to the obtaining of antimicrobial activity in front of Gram-positive and Gram-negative strains, indicating, therefore, inhibitory activity of wide spectrum of action. Taking into account the results presented here, it is emphasized that HEG promoted inhibition on the growth of bacteria of high pathogenicity, considered important etiological agents of various types of infections in humans and animals, of difficult inhibition and that develop frequently resistance to various classes of antimicrobials (Souza et al., 2015).

Campêlo et al. (2015), also investigated the antimicrobial activity of hydroalcoholic extracts from the geopropolis of the bee *Partamona cupira* collected in the semiarid region of

the State of Rio Grande do Norte, in northeastern Brazil. In this study, the tests were performed through the agar diffuser test and bacteria were tested in different concentrations of the extract. The authors found that the investigated extract, in the concentration of 100 µg.mL^{-1} had activity demonstrated effective antimicrobial action, promoting the inhibition of the growth of *E. aerogenes*, *E. coli* and *S. aureus*. Thus, it is possible to notice results similar to those found in our study regarding the capacity as to the antibacterial action of the geopropolis produced by the bee *Partamona cupira*. These results may represent an important advance in the search for alternatives for the development of new antibiotics based on natural products.

4.6 *In vivo* healing activity

The healing process is a complex sequence of cellular and biochemical events with the purpose of restoring tissue integrity after trauma. The bioactive compounds present in propolis are able to modulate these events and interfere to a greater or lesser degree in the healing process (Kapoor and Appleton, 2005). Here, we evaluate the effect of treating experimental skin wounds treated with geopropolis cream by comparing the results obtained in morphometric, macroscopic and histological evaluation with base cream without the addition of geopropolis.

The HEG was found to have a positive influence on the healing of experimental skin wounds in rats. This can be justified by the finding of faster closure in relation to the control group. The histological parameters of those treated with cream containing geoprolis the wounds on the 21 day, proved the resolution of the healing process, since in this period there was an increase in the density of fibroblasts and collagen fibers, and complete restoration of the epithelial layer that was reepithelialized. While the wounds of the control group needed more time for resolution of the healing process.

In the composition of HEG there are substances described in the literature as capable of promoting a beneficial effect in the treatment of skin wounds. The phenolic compounds and their derivatives stand out, especially the flavonoids that positively favor the healing process. The flavonoid quercetin has remarkable healing activity as it promotes reepithelialization (Suntar et al., 2010). Kaempferol acts in the promotion of maximum traction force of wound edges, favors reepithelialization and promotes keratinocyte migration (Petpiroon et al., 2015; Özay et al., 2019). Chalcones have anti-inflammatory properties and the ability to reduce carrageenan-induced mice paw edema (Viana et al., 2003). Apigenin

flavonoid also presents anti-inflammatory activity by reducing inflammatory edema of the rat, besides presenting a favorable effect on wound healing since through the evaluation of the contraction values of wounds treated with this compound it was evident that there was acceleration in healing speed, besides the significant increase in granulation tissue of the number of capillaries, fibroblasts and collagen when compared to animal control granuloma (Manivannan, 2016). Thus, the presence of various compounds identified in HEG by the technique of HPLC-DAD-ESI-MS may explain its healing action found by us.

5. Conclusions

It can be concluded that the hydroalcoholic extract of *Partamona cupira* bee's geopropolis presented in its composition phenolic compounds with high biological activity such as chalcones, flavones and flavonols, as well as revealed high antioxidant activity and promoted genoprotective effect by reducing damage to DNA of L929 fibroblasts injured by H_2O_2. In the experimental conditions used, it was evidenced that the extract showed antimicrobial activity promoting inhibition of *S. aureus*, *S. pyogenes*, *E. coli* and *E. aerogenes*. The application of the cream containing geopropolis showed a positive influence on experimental skin wound healing in Wistar rats, by promoting faster wound closure and reepithelialization in relation to the control group, in addition to a less intense inflammatory reaction, increased fibroblastic proliferation and collagen deposition.

The results obtained in this work open perspectives for further research, such as isolation of the bioactive compounds present in the geopropolis, investigations on the mechanisms of action of the compounds, their subsequent use as a raw material in functional foods, medicines and cosmetics.

Conflicts of Interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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Percentage of contribution of each author in the manuscript

Jael Soares Batista – 10%

Jardel Bezerra da Silva – 10%

Kizzy Millenn de Freitas Mendonça Costa – 10%

Tiago Da Silva Teófilo – 10%

Natanael Silva Félix – 10%

Francisco Herbeson Aquino Silva – 10%

Luciana Cristina Borges Fernandes – 10%

Wanderson Lucas Alves Dos Santos – 10%

Wesley Lyeverton Correia Ribeiro – 10%

Geysa Almeida Viana – 10%