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Effect of extracts of amazonian bee propolis on *Xanthomonas axonopodis* pv. *passiflorae* in the State of Pará-Brazil

Efeito de extratos de própolis apícola amazônica sobre *Xanthomonas axonopodis* pv. *passiflorae* no Estado do Pará-Brasil

Efecto de extractos de propóleo de abeja amazónica sobre *Xanthomonas axonopodis* pv. *passiflorae* en el Estado de Pará-Brasil

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Abstract

The yellow passionfruit (*Passiflora edulis* Sims f. *flavicarcarpa* Deg.) is important culture in Brazilian Amazon agriculture, especially in the state of Pará. But its cultivation has been suffering the reduction of its areas and productivity due the diseases caused by bacteria, where

chemical control, sometimes does not present the expected results. The propolis of Africanized bees (*Apis mellifera* L.) is an important natural antibiotic for the control of undesirable microorganisms of plants and animals. The present work aimed at the *in vitro* study of the antibiotic activity of different propolis extracts of Africanized bees from two different locations in the state of Pará in the agent that causes the passionfruit bacterial blight (*Xanthomonas axonopodis* pv. *passiflorae*). A factorial analysis of three factors was performed: origin X solvent X concentration. It was verified that the concentrations of 0.5% were statistically superior to the others, with average growth inhibition power of 86%, and the propolis extract from an apiary in Santa Izabel do Pará, Pará, Brazil, obtained in ethanol at 80%, was statistically different and superior for the inhibitory effect of the growth of colony forming units (CFU) of *X. axonopodis* pv. *passiflorae*.

Keywords: Beekeeping; Passionfruit; Amazon; Phytopathogens.

Resumo

O maracujá-amarelo (*Passiflora edulis* Sims f. *flavicarcarpa* Deg.) é uma cultura importante na agricultura da Amazônia brasileira, especialmente no estado do Pará. Mas o seu cultivo tem sofrido uma redução de suas áreas e produtividade devido às doenças causadas por bactérias, em que o controle químico, às vezes, não apresenta os resultados esperados. A própolis de abelhas africanizadas (*Apis mellifera* L.) tem se mostrado um importante antibiótico natural no controle de microrganismos indesejáveis de plantas e animais. O presente trabalho teve como objetivo o estudo *in vitro* da atividade antibiótica de diferentes extratos de própolis de abelhas africanizadas de duas diferentes localidades do estado do Pará sobre o agente causador da mancha bacteriana do maracujá (*Xanthomonas axonopodis* pv. *passiflorae*). Foi realizada uma análise fatorial de três fatores: origem X solvente X concentração. Verificou-se que as concentrações de 0,5% foram estatisticamente superiores às demais, com poder de inibição do crescimento médio de 86%, e o extrato de própolis do apiário de Santa Izabel do Pará, Pará, Brasil, obtido em etanol a 80%, demonstrou efeito inibitório estatisticamente diferente e superior do crescimento de unidades formadoras de colônias (UFC) de *X. axonopodis* pv. *passiflorae*.

Palavras-chave: Apicultura; Maracujá; Amazônia; Fitopatógeno.

Resumen

El maracuyá amarillo (*Passiflora edulis* Sims f. *flavicarcarpa* Deg.) es una cultura importante en la agricultura de la Amazonía brasileña, especialmente en el estado de Pará. Pero su cultivo

ha sufrido una reducción de sus áreas y productividad debido enfermedades causadas por bacterias, en las que el control químico, en ocasiones, no presenta los resultados esperados. Se ha demostrado que el propóleo de abejas africanizadas (*Apis mellifera* L.) es un importante antibiótico natural en el control de microorganismos indeseables de plantas y animales. El presente trabajo tuvo como objetivo estudiar *in vitro* la actividad antibiótica de diferentes extractos de propóleo de abejas africanizadas de dos localizaciones distintas del estado de Pará sobre el agente causante de la mancha bacteriana de la maracuyá (*Xanthomonas axonopodis* pv. *passiflorae*). Se realizó un análisis factorial de tres factores: origen X solvente X concentración. Se encontró que las concentraciones de 0.5% fueron estadísticamente superiores a las demás, con un poder inhibidor del crecimiento promedio de 86%, y el extracto de propóleo del apiario de Santa Izabel do Pará, Pará, Brasil, obtenido en etanol al 80%, demostró un efecto inhibidor estadísticamente diferente y superior sobre el crecimiento de unidades formadoras de colonias (UFC) de *X. axonopodis* pv. *passiflorae*.

Palabras clave: Apicultura; Maracujá; Amazonía; Fitopatógeno.

1. Introduction

The use of natural products to control bacterial diseases in plants is considered an interesting alternative to synthetic bactericides and fungicides due to the low negative impact on the environment (Ordóñez et al., 2011).

An alternative to the use of agrochemicals is the use of integrated management of plant diseases with agroecological focus, which includes biological control, cultural control, the use of resistant cultivars, and the use of natural products with resistance-inducing activity and/or with direct antimicrobial activity. This last form of control, fit the use of plant and microbial extracts in plants (Trusheva et al., 2011).

Bee products have great relevance in the world scenario, much of it is due to the fact that they present biological activities, and propolis is produced by bees and has been reported in the literature as an agent that promotes antibacterial activity (Trusheva et al., 2011).

The morphological characteristics of propolis are reported by Bankova, Popova e Trusheva (2014) (Bankova et al., 2014), and biological activities are generally attributed to the phenolic compounds found in this product. Gülçin et al. (2010) (Gülçin et al., 2010), and Szliszka et al. (2011) (Szliszka et al., 2011), citing that green propolis, for example, has an immuno-modulator, anti-tumor, and chemopreventive properties. Also, antioxidant activities can also be seen in this product (Miguel et al., 2010).

Propolis has been the subject of studies related to agricultural activities, according to Pereira et al. (2017) (Pereira et al., 2017), the authors reported *in vitro* bioherbicidal activity of the geopropolis of native bees *Melipona subnitida* in grassland weeds. Propolis has also demonstrated antimicrobial action in pathogenic bacteria in livestock activities (Al-Abbadi et al., 2015; Heimbach et al., 2016; Kalogeropoulos et al., 2009), as well as in phytopathogenic bacteria of importance for agriculture (Pereira et al., 2016; Pereira[1] et al., 2016).

Another important factor of propolis is the chemical composition that is linked to the region of production, availability of sources to collect plant resins, queen bee genetic variability, and seasonality, the variation of chemical composition directly influences the biological responses of propolis (Toreti et al., 2013).

Propolis significantly reduces the incidence and severity of various phytopathogenic agents, which cause disease in plants, and have beneficial effects on plants. Studies on the use of propolis are just beginning and other studies in othercrops still need to be conducted to increase the knowledge of propolis use in agriculture (Pereira[1] et al., 2016).

Among the diseases associated with passionfruit cultivation, bacteriosis caused by *Xanthomonas axonopodis* pv. *passiflorae*, has been highlighted due to the damage caused to the crop, especially in regions with temperature and humidity favorable to the occurrence of the disease and because it is a disease of difficult control (Alessandra Keiko Nakasone Ishida, 2009).

Studies with extracts of propolis marketed in Brazil showed pronounced antimicrobial activity against Gram-positive bacteria, and less evident activity against Gram-negative (Packer & Da Luz, 2007).

In view of the above, the objective of this work was to evaluate the antibacterial activity of different propolis extracts, from two different locations in the state of Pará, Brazil, against *X. axonopodis* pv. *passiflorae*.

2. Methodology

Material collection in the field, *in vitro* application, analysis, and organization of information occurred in 12 months from June 2014.

Apiaries were selected with hives inhabited by *Apis mellifera* L. bees (Africanized) for propolis collection, distributed in a region of the Amazonian biome, located in distinct areas of plant predominance, in the state of Pará, Brazil. The apiary in the municipality of Santa Izabel do Pará, Pará, Brazil, had nearby growing area of cultures native to the Amazon: açaí

(*Euterpe oleraceae* Mart.), and cacao (*Theobroma cacao* L.); and in Curuçá, Pará, Brazil, the hives were distributed in Amazonian forest area in the vicinity of small-scale cropping areas of a long and short cycle of family farmers.

2.1 Collecting and preparation of extracts

The method of collecting propolis was the scraping of internal parts of *Langstroth* hives. After the impurities were separated, each sample was packed in a closed container, protected from light, and cooled below 0 °C. Each sample was weighed, ground, packed into Erlenmeyer flasks to which solvents were added in increasing order of polarity: hexane, ethyl acetate, and ethanol at 80%. Subsequently, a 2.5 g fraction of each of the propolis extracts, which were previously dehydrated to the pasty form, was separated into sterilized becker and 25 ml of each of their respective solvents previously used to solubilize the extracts were added, a 10% concentration of propolis extract was obtained. All were packed in Erlenmeyer flasks and identified.

2.2 Chemical Analysis

The phenolic profiles were obtained via high-performance liquid chromatography/diode array detection/** (HPLC/DAD/EM-EM). The profile analysis of the ethanolic extracts was performed using a Thermo-brand high-performance liquid chromatography with an automatic injector, a 20-µL sampling handle, and a quaternary pump. It was coupled with a diode array detector and a mass spectrometer equipped with an electrospray ionization (ESI) source and an ion-trap mass analyzer. The equipment was operated at room temperature (25 ± 2 °C), and the chromatographic data were obtained and processed by the Xcalibur software. The C18 chromatographic column used was reversed phase (150 x 2.1 mm) and had a particle size of 1.9 µm (Thermo Scientific Hypersil GOLD). The chromatographic method was based on that of (NOVÁKOVÁ; Solich; Solichová, 2008), with minor modifications. The mobile phase consisted of 0.1% aqueous formic acid solution (A) and acetonitrile (B). The elution gradient started at a ratio of 95:5 at a flow rate of 0.32mL min-1111. The concentration of "A" decreased to the 50:50 condition in 5 min, and from 6 min onward it gradually returned to the initial condition of 95:5, which required 20 min. It remained in this condition for a further 2 113 min for the initial conditions to be restored.

Compound detection was performed using a DAD detector (operating at 210, 260, 300, and 325 nm) and a mass spectrometer with an ESI source operating in the negative mode (capillary temperature 350 °C, capillary voltage 2.5 kV, cone voltage 5 kV). He and N2 were used as the collision gas and nebulizer gas 70 (arbitrary unit), respectively. The compounds present in the samples were identified based on the retention time, absorption spectra, and co-chromatography, which were comparable with the standards and mass spectra results, which helped in confirming the chemical structures of the compounds.

2.3 Bacterial strain

The bacteria *X. axonopodis* pv. *passiflorae*, from the yellow passionfruit culture (*Passiflora edulis* Sims f. *flavicarpa* Deg.), used in this work were obtained in the Collection of Phytopathogenic Bacteria Cultures of Embrapa Amazônia Oriental, Belém, Pará, Brazil. The *X. axonopodis* pv. *passiflorae*, were reactivated from stock in culture medium 523 (Kado; Heskett, 1970) during 18-24 h at 28 ºC.

2.4 Assay

The analysis of antibacterial activity was performed in plate by determining the Minimum Inhibitory Concentration (MIC). To evaluate the *in vitro* effect of the propolis extracts on bacterial growth, these were incorporated into culture medium 523 in the concentration of 0.5%. Five replicates were used for each treatment, where each plate represented one replicate. The extracts were added to the still liquid culture medium at a temperature of 50-70 °C, carefully homogenized under rotary movements distributed in Petri dishes in a volume of 20 ml. After solidification of the medium, 100 µl of each bacterial suspension aliquots were placed and adjusted to Abs600 = 0.3 in 10^{-6} dilution and scattered with Drigalski strap, previously sterilized by buckling. As control, the culture medium was used without adding any extract. The cultures were incubated at a temperature of 28 ± 2 °C. The growth of *X. axonopodis* pv. *passiflorae* was measured 48 h after cultivation.

2.5 Experimental design and statistical analysis

The Lilliefors and Shapiro-Wilk tests were applied to verify the normality, followed by an analysis of variance, and Tukey mean tests ($\alpha = 0.5$). In all tests, $\alpha = 0.05$ was used as the significance value.

The data were evaluated by factorial analysis of three factors:

- Origin of propolis (2) X Solvents (3) X Concentrations (4);

Thus, the plots were constituted by treatments with extracts of propolis from two different origins in the state of Pará (Santa Izabel do Pará, and Curuçá), obtained with three different solvents (hexane, ethyl acetate and ethanol at 80%) at the concentration of 0.2%; 0.3%; 0.4%; and 0.5%. Treatment with only water was used to serve as a control, totaling 25 treatments.

The experimental design was completely randomized (DIC), and the data, with three repetitions, were subjected to analysis of variance, by the F test, using the Tukey test for comparison of means and the t-test for the coefficients of the equations. regression, with the aid of the SISVAR 5.3 software application (Ferreira, 2010) and electronic spreadsheet.

3. Results and discussion

3.1 Chemical composition

The propolis samples showed little difference in their chemical composition in terms of phenolic compounds (Table 1), this may be related to the fact that the collections were carried out in areas of municipalities in the same region, in the Northeast of Pará, sharing both samples, in its composition of variability one of the compounds.

Table 1. Phenolic compounds identified in the propolis samples of Apis mellifera.

Origin of samples	Extraction type	Compounds	[M-H]- m/z	M2
Santa Izabel-PA	Soxhlet extraction	3,4- dihydroxybenzoic	[153]	[153] 109
		Kaempferol	[285]	[285] 217, 151
Curuçá-PA	Soxhlet extraction	Gallic acid	[169]	[169] 125
		3,4- dihydroxybenzoic	[153]	[153] 109

Source: Authors (2020).

The chemical composition of propolis is variable not only due to the variability of plant sources but also depending on the bee species, lighting, altitude, and food availability.

However, the general composition of most samples of crude propolis is similar (Toreti et al., 2013).

In general, the chemical composition of propolis is complex and can be formed from volatile compounds of low molecular weight to compounds of greater mass and high polarity, such as phenolic compounds (Bertrams et al., 2013; Li et al., 2012). Other scientific reports have shown that the biological activities presented by propolis extracts are directly linked to their chemical composition (Catchpole et al., 2015; Silva et al., 2012; Szweda et al., 2015), an example is a work of Castro et al. (2014) (Castro et al., 2014), in which the antioxidant activity of six samples of propolis was evaluated and found that the fractions that showed the highest concentration of compounds such as caffeic acid, benzyl ester of caffeic acid and quercetin showed the highest antioxidant activities in all methods analyzed, similar results can be observed in the work of (Siripatrawan et al., 2013) with extracts of propolis from propolis collected in Thailand.

3.2 Inhibitory effect on Xanthomonas axonopodis pv. passiflorae

The propolis of the apiaries in municipalities of Curuçá and Santa Izabel do Pará, was extracted sequentially with three solvents of increasing polarity: hexane, ethyl acetate, and ethanol at 80%. The solvent was removed by rotary evaporator under reduced pressure to obtain the propolis extracts at the pulp point for each of the solvents which were weighed to verify the individual yield of the extraction method.

For Kalogeropoulos et al. (2009) (Kalogeropoulos et al., 2009), the propolis produced by bees is generally purified by extraction with different solvents to remove the wax and organic residues to preserve the polyphenol fraction, which contains most of the bioactive present in the propolis. Considering that propolis is a potent natural antibiotic, and this action seems to be more related to the joint action of its compounds than the isolated action of each of them (Bogdanov, 2017), the withdrawal of bioactive with the use of solvents sequentially may show a more efficient extraction, presenting different results in different polarities.

The analysis of variance for colony forming unit (CFU) of *X. axonopodis* pv. *passiflorae* was applied under the effect of extracts of propolis from different sources, obtained with three different solvents, in four different concentrations. The analysis of variance showed significant effect on the contrast between the control and the applied treatments (Table 2).

Table 2. Summary of the analysis of variance for colony-forming unit (CFU) of *Xanthomonas axonopodis* pv. *passiflorae* under the effect of propolis extracts from different sources, obtained with three different solvents, in four concentrations.

V.F.[1]	**D.F.[2]**	**M.S.[3]**
Witness vs. Other treatments	1	6965.80**
Source	1	110.21[n.s.]
Solvent	2	5191. 28**
Concentration	3	17848.21**
Source x Solvent	2	453.96**
Source x Concentration	3	143.74 [n.s.]
Solvent x Concentration	6	1544.41**
Source x Solvent x Concentration	6	148.43 [n.s.]
Error	100	73.92
C.V. [4] (%)		19.46
Mean		44.18

[1]V.F. – Variation factor. [2]D.F. – Degrees of freedom. [3]M. S. –Mean squares. [4]C.V. – Coefficient of variation. **, * and [n.s] indicate significance at 1.5% and not significant. Source: Authors (2020).

The significance for the Source x Solvent interaction (Table 3) may be the result of differences in the dissolution of propolis with the evaluated solvents. It is verified that the 80% ethanol solvent caused greater reduction of bacterial growth for both propolis studied.

Table 3. Growth of *Xanthomonas axonopodis* pv. *passiflorae* (CFU) (mean ± standard deviation) under the effect of propolis extracts from different sources, obtained with three different solvents, in four concentrations.

Source	**Solvent**		
	Hexane	**Ethyl Acetate**	**Ethanol 80%**
Santa Izabel do Pará	50.95±15.73aA**	49.3±30.45aA**	24.90±23.13bB**
Curuçá	46.95±20.51aA**	49.8±31.84aA**	34.15±24.7bA**
Control	80.20±7.19		

Means followed by distinct letters in the column (upper case) and in the line (lower case) differ from each other by the Tukey test at 5%. ** indicates significance at the 1% level (Test F) for the contrast between the control and the respective treatment. Source: Authors (2020).

These results corroborate with that reported by Fábio et al. (2014) (Fábio et al., 2014), who observed direct effect of the antibacterial activity of propolis on *X. axonopodis* pv. *phaseoli* of the bean by the ethanolic extract of propolis, where the propolis promoted a

drastic reduction in the bacterium development, showing linear effect with greater activity as concentration increase.

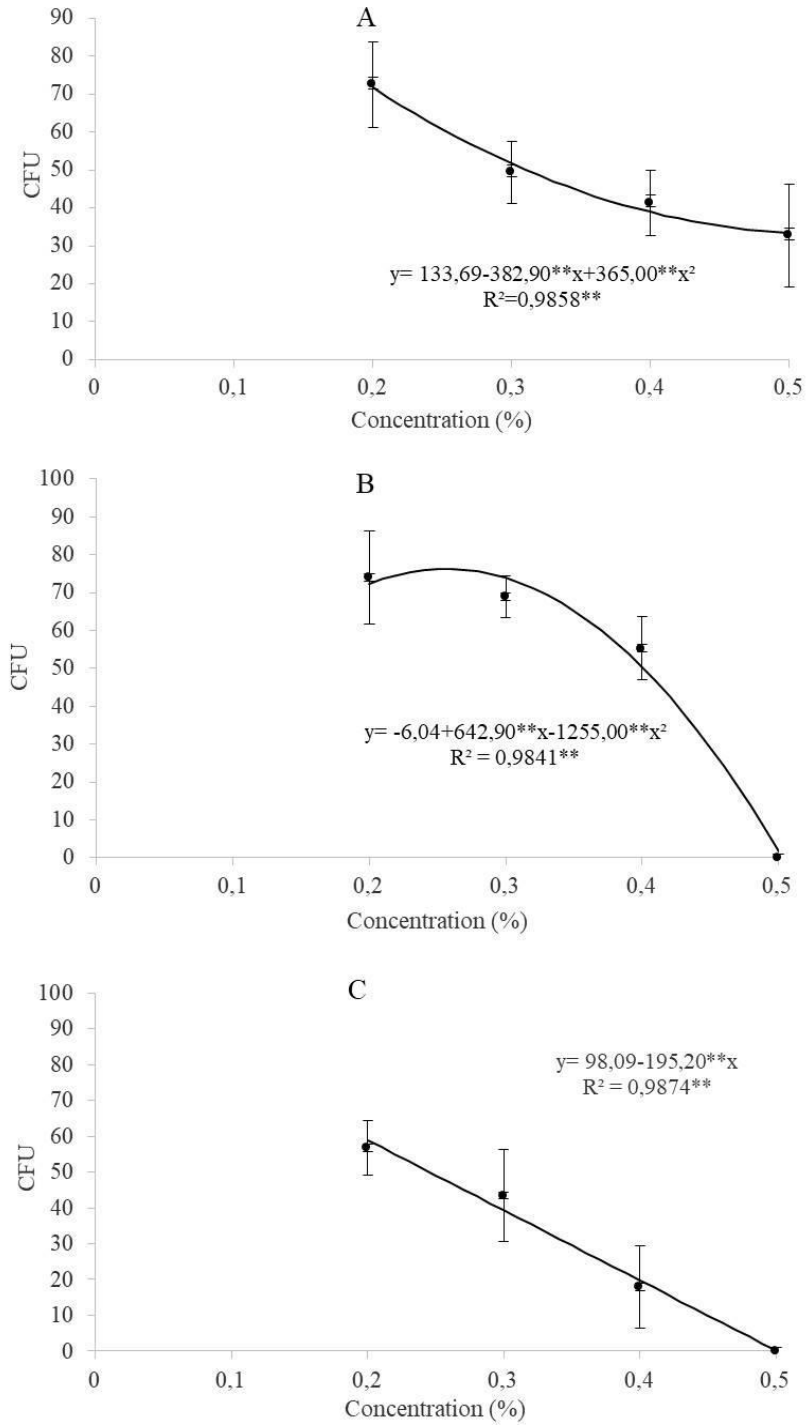
However, the number of CFUs was significantly lower with the propolis of Santa Izabel do Pará (24.90 CFU) when compared to that of Curuçá (34.15 CFU), for this same solvent, equivalent to a mean reduction of growth of approximately 69% and 57%, respectively. These results suggest that the extracts obtained with high polarity solvents were efficient for *X. axonopodis* pv. *passiflorae*, thus, we can state that the bioactives of the propolis tested to have an efficiency in the inhibition of CFUs in the higher polarities of the solvents used for extraction. In this case, the 80% ethanolic extract of propolis presented better results, from the propolis of the apiary in the municipality of Santa Izabel do Pará, possibly because the allelochemicals with greater effect in the bacterium are concentrated in extracts of polarity intermediate to greater.

The difference between polar and nonpolar substances is in the presence or absence of electronegative atoms in their structures, which causes differences in the intermolecular forces acting on them. In part of the nonpolar substances, the attraction of the molecules is weaker and this facilitates the movement of these molecules, normally having extremely low melting and boiling points. In polar substances, due to the existence of regions with different electronic densities, a stronger force of attraction acts on the molecules, which hinders the movement of these molecules and prevents them from reaching the gas state so easily.

Because propolis is a very complex mixture of substances of varied polarity it is difficult to find a single solvent that extracts all the components of interest, so, in the scientific literature, several solvents are available to obtain extracts of propolis. The most commonly used solvent is ethanol at concentrations ranging from 30% v/v in water to absolute ethanol (Cunha et al., 2004).

Dependence was observed between the effects of the solvent and the concentrations used (Table 1), by adjusting the CFU curves x Concentration for each solvent used (A: hexane; B: ethyl acetate; C: ethanol 80%) (Figure 1). The reduction rate of bacterial growth with increasing concentration was linear (195.20 CFU for each increment of 1%) when using ethanol at 80%, whereas for other solvents this was variable, as can be deduced from the models adjusted.

Figure 1. *Xanthomonas axonopodis* pv. *passiflorae* Colony-Forming Units (CFU) means in the function of the concentration of the extract obtained with the solvents hexane (A), ethyl acetate (B) and, ethanol at 80% (C).



Vertical bars represent the upper and lower limits corresponding to the value of a standard deviation for plus and minus. * and ** indicate significance at 5% and 1%, respectively.
Source: Authors (2020).

For the hexane solvent, the CFU reduction rate varied between 236.90 and 17.90, while for ethyl acetate this varied between 140 and 612.1 CFU% between the lowest and highest concentrations evaluated. For the first, the effect was higher at lower concentrations but showed a maximum reduction of approximately 58% in bacterial growth about the

control (80.2 CFU). When the hexane solvent was used, there was a small reduction in the lowest concentrations of the extract, but in the highest concentration, the bacterial population was reduced to 1.67 CFU, on average, corresponding to a reduction of approximately 98% of the bacterial population. The adaptation to the new environment may have influenced the bacterial growth response pattern, particularly when the hexane solvent was used, in which there appears to be a principle of stabilization at the population level. An evaluation of the bacterial growth in function of time for each concentration can help to better understand this phenomenon.

Considering the estimated concentration to reduce bacterial growth by 50%, ethanol solvent was more effective (0.30%), followed by hexane (0.39%) and ethyl acetate (0.43%). Also, ethanol at 80% was the only solvent of which there was total inhibition of bacterial growth at the maximum concentration evaluated (0.5%). In a previous preparatory test, a total bacterial population reduction at a concentration of 1% of the extract was observed for all solvents.

Kameyama et al. (2008) (Kameyama et al., 2008) reported that the alcoholic extract of propolis exerts antimicrobial activity on minimally processed carrot contaminants, with a concentration of 0.4% (m/v) being the most suitable for use.

The use of propolis has already been described for other bacteria, extract of propolis has been tested from the commercial product at the concentration of 10% in eight different phytobacteria: *Pseudomonas syringae* pv. *tomato*, *P. corrugata*, *Clavibacter michiganensis* subsp. *michiganensis*, *Erwinia carotovora* subsp. *carotovora*, *Xanthomonas campestris* pv. *vesicatoria*, *X. translucens* pv. *undulosa*, *X. axonopodis* pv. *phaseoli* and *X. campestris* pv. *campestris*. These authors were able to inhibit markedly the multiplication of these bacteria.

Féas et al. (2014) (Feás et al., 2014) recommend propolis as a sanitizing agent. In relation to the average reduction of aerobic and psychotropic mesophiles and fecal coliform, the propolis extract was more efficient in the microbiological reduction than the commercial sodium hypochlorite product.

The beekeeping propolis extract of the Amazon demonstrated the potential to become an agroecological biocontrol alternative to the traditional agricultural practices for the control of diseases caused by phytopathogenic bacteria, as is the case of the passionfruit bacterial blight caused by *X. axonopodis* pv. *passiflorae*, making the production of this food more sustainable, bringing fewer risks to human health because it is a product of natural source.

The inhibitory effects on the development of *X. axonopodis* pv. *passiflorae* were more pronounced when the propolis concentration was increased in the test solutions, and the

greatest inhibitory effects were observed in the propolis from the city of Santa Izabel do Pará when used ethanol at 80%. In general, higher inhibitory effects were obtained with the 80% ethanol solvent independent of the origin, with inhibition of 50% and 100% of the CFU development at concentrations of 0.30% and 0.50% of the extract. Higher effects at lower concentrations were verified with the use of the hexane solvent and at high concentrations with the use of ethyl acetate.

4. Final Considerations

Studies to evaluate the *in vivo* effect are necessary to verify the use in the field, and considering that the antimicrobial activity observed in the present work may be related to the botanical origin of propolis, other studies are necessary to identify the bioactive compounds present in the Amazonian propolis of the state of Pará.

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Conflict of interests

There are no conflicts of interest

Referências

Al-Abbadi, A. A., Ghabeish, I. H., Ateyyat, M. A., Hawari, A. D., & Araj, S. E. A. (2015). A comparison between the anti-microbial activity of native propolis and the anti-microbial activity of imported ones against different health microbes. *Jordan Journal of Biological Sciences*, *8*(1), 65–70. https://doi.org/10.12816/0026951

Ishida, A., K., N., Halfeld-Vieira, B., D., A. (2009). Mancha-Bacteriana do Maracujazeiro (Xanthomonas axonopodis pv. passiflorae): Etiologia e Estratégias de Controle. *Embrapa Amazônia Oriental*. https://www.infoteca.cnptia.embrapa.br/bitstream/doc/874307/1/maracujazeiro.pdf

Bankova, V., Popova, M., & Trusheva, B. (2014). Propolis volatile compounds: Chemical diversity and biological activity: A review. *Chemistry Central Journal*, *8*(1), 28. https://doi.org/10.1186/1752-153X-8-28

Bertrams, J., Müller, M. B., Kunz, N., Kammerer, D. R., & Stintzing, F. C. (2013). Phenolic compounds as marker compounds for botanical origin determination of German propolis samples based on TLC and TLC-MS. *Journal of Applied Botany and Food Quality*, *86*(1), 143–153. https://doi.org/10.5073/JABFQ.2013.086.020

Castro, C., Mura, F., Valenzuela, G., Figueroa, C., Salinas, R., Zuñiga, M. C., Torres, J. L., Fuguet, E., & Delporte, C. (2014). Identification of phenolic compounds by HPLC-ESI-MS/MS and antioxidant activity from Chilean propolis. *Food Research International*, *64*, 873–879. https://doi.org/10.1016/j.foodres.2014.08.050

Catchpole, O., Mitchell, K., Bloor, S., Davis, P., & Suddes, A. (2015). Antiproliferative activity of New Zealand propolis and phenolic compounds vs human colorectal adenocarcinoma cells. *Fitoterapia*, *106*, 167–174. https://doi.org/10.1016/j.fitote.2015.09.004

Cunha, I. B. S., Sawaya, A. C. H. F., Caetano, F. M., Shimizu, M. T., Marcucci, M. C., Drezza, F. T., Povia, G. S., & Carvalho, P. D. O. (2004). Factors that influence the yield and composition of Brazilian propolis extracts. *Journal of the Brazilian Chemical Society*, *15*(6), 964–970. https://doi.org/10.1590/S0103-50532004000600026

Fábio, J., Jonas, M., Gabriela, S., & Mailis, A. (2014). *Indução de faseolina em feijão e na atividade antibacteriana sobre Xanthomonas axonopodis pv. phaseoli pelo extrato etanólico de própolis*. Cadernos de Agroecologia. http://revistas.aba-agroecologia.org.br/index.php/cad/article/view/15576/10082

Feás, X., Pacheco, L., Iglesias, A., & Estevinho, L. M. (2014). Use of propolis in the

sanitization of lettuce. *International Journal of Molecular Sciences*, *15*(7), 12243–12257. https://doi.org/10.3390/ijms150712243

Gülçin, I., Bursal, E., Şehitoğlu, M. H., Bilsel, M., & Gören, A. C. (2010). Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. *Food and Chemical Toxicology*, *48*(8–9), 2227–2238. https://doi.org/10.1016/j.fct.2010.05.053

Heimbach, N. D. S., Ítavo, C. C. B. F., Leal, C. R. B., Ítavo, L. C. V., Silva, J. A. Da, Silva, P. C. G., Rezende, L. C. De, & Gomes, M. D. F. F. (2016). Propolis extraction residue like bacterial inhibitor "in vitro." *Revista Brasileira de Saude e Producao Animal*, *17*(1), 65–72. https://doi.org/10.1590/s1519-99402016000100007

Kalogeropoulos, N., Konteles, S. J., Troullidou, E., Mourtzinos, I., & Karathanos, V. T. (2009). Chemical composition, antioxidant activity and antimicrobial properties of propolis extracts from Greece and Cyprus. *Food Chemistry*, *116*(2), 452–461. https://doi.org/10.1016/j.foodchem.2009.02.060

Kameyama, O., Abrão Júnior, J., Maria de Assis Teixeira, J., José de Andrade, N., Paula Rodrigues Minin, V., & dos Santos Soares, L. (2008). Extrato de própolis na sanitização e conservação de cenoura minimamente processada. *Ceres*, *55*(3). http://www.ceres.ufv.br/ojs/index.php/ceres/article/view/3307

Li, Y. J., Xuan, H. Z., Shou, Q. Y., Zhan, Z. G., Lu, X., & Hu, F. L. (2012). Therapeutic effects of propolis essential oil on anxiety of restraint-stressed mice. *Human and Experimental Toxicology*, *31*(2), 157–165. https://doi.org/10.1177/0960327111412805

Miguel, M. G., Nunes, S., Dandlen, S. A., Cavaco, A. M., & Antunes, M. D. (2010). Phenols and antioxidant activity of hydro-alcoholic extracts of propolis from Algarve, South of Portugal. *Food and Chemical Toxicology*, *48*(12), 3418–3423. https://doi.org/10.1016/j.fct.2010.09.014

Ordóñez, R. M., Zampini, I. C., Moreno, M. I. N., & Isla, M. I. (2011). Potential application of Northern Argentine propolis to control some phytopathogenic bacteria. *Microbiological*

Research, *166*(7), 578–584. https://doi.org/10.1016/j.micres.2010.11.006

Packer, J. F., & Da Luz, M. M. S. (2007). Evaluation and research method for natural products inhibitory activity. *Brazilian Journal of Pharmacognosy*, *17*(1), 102–107. https://doi.org/10.1590/s0102-695x2007000100019

Pereira, D. S., Abrantes, M. R., Coelho, W. A. C., Freitas, M. O., Freitas, C. I. A., & Silva, J. B. A. da. (2016). Potencial antibiótico da própolis apícola Potiguar em bactérias de importância veterinária. *Revista Verde de Agroecologia e Desenvolvimento Sustentável*, *11*(3), 151. https://doi.org/10.18378/rvads.v11i3.4377

Pereira, D. S., De Holanda-Neto, J. P., De Oliveira, M. S., Pereira, N. S., Maracajá, P. B., & Souza Filho, A. P. D. S. (2017). Phytotoxic potential of the geopropolis extracts of the jandaira stingless bee (Melipona Subnitida) in weeds. *Revista Caatinga*, *30*(4), 876–884. https://doi.org/10.1590/1983-21252017v30n407rc

Pereira, C. S., Daróz Matte, W., Henrique, P., & Venâncio, B. (2016). Aplicação de extrato de própolis na agricultura. In *Revista De Ciências Agroambientais*, 14(1). https://periodicos.unemat.br/index.php/rcaa/article/view/1421

Silva, J. C., Rodrigues, S., Feás, X., & Estevinho, L. M. (2012). Antimicrobial activity, phenolic profile and role in the inflammation of propolis. *Food and Chemical Toxicology*, *50*(5), 1790–1795. https://doi.org/10.1016/j.fct.2012.02.097

Siripatrawan, U., Vitchayakitti, W., & Sanguandeekul, R. (2013). Antioxidant and antimicrobial properties of Thai propolis extracted using ethanol aqueous solution. *International Journal of Food Science & Technology*, *48*(1), 22–27. https://doi.org/10.1111/j.1365-2621.2012.03152.x

Szliszka, E., Zydowicz, G., Janoszka, B., Dobosz, C., Kowalczyk-Ziomek, G., & Krol, W. (2011). Ethanolic extract of Brazilian green propolis sensitizes prostate cancer cells to TRAIL-induced apoptosis. *International Journal of Oncology*, *38*(4), 941–953. https://doi.org/10.3892/ijo.2011.930

Szweda, P., Gucwa, K., Kurzyk, E., Romanowska, E., Dzierżanowska-Fangrat, K., Zielińska Jurek, A., Kuś, P. M., & Milewski, S. (2015). Essential Oils, Silver Nanoparticles and Propolis as Alternative Agents Against Fluconazole Resistant Candida albicans, Candida glabrata and Candida krusei Clinical Isolates. *Indian Journal of Microbiology*, *55*(2), 175–183. https://doi.org/10.1007/s12088-014-0508-2

Toreti, V. C., Sato, H. H., Pastore, G. M., & Park, Y. K. (2013). Recent progress of propolis for its biological and chemical compositions and its botanical origin. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 13. https://doi.org/10.1155/2013/697390

Trusheva, B., Popova, M., Koendhori, E. B., Tsvetkova, I., Naydenski, C., & Bankova, V. (2011). Indonesian propolis: Chemical composition, biological activity and botanical origin. *Natural Product Research*, *25*(6), 606–613. https://doi.org/10.1080/14786419.2010.488235

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