Anales de Biología 45: 33-41, 2023
http://dx.doi.org/10.6018/analesbio.45.04
ISSN/eISSN 1138-3399/1989-2128

ARTICLE

# Evaluation of *Stryphnodendron polyphyllum* Mart. pollen as antioxidant and antibacterial potential

Rafael Nogueira Oliveira Albuquerque[1], Carlos Henrique Gomes Martins[2], Jonathan Henrique de Souza[2], Verônica de Melo Sacramento[1], Dario Alves de Oliveira[1], Afrânio Farias de Melo-Júnior[1], Elytania Veiga Menezes[1], Luciano Fernandes Souza[3] & Vanessa de Andrade Royo[1]

1 Natural Products Laboratory, State University of Montes Claros, Montes Claros, Minas Gerais, Brazil.
2 Laboratory of Antimicrobial Testing, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil.
3 Cooperative of Beekeepers and Family Farmers of the North of Minas, Bocaiuva, Minas Gerais, Brazil.

Correspondence
V.A. Royo
E-mail: vanroyo31@gmail.com
**Received:** 4 July 2022
**Accepted:** 5 January 2023
**Published on-line:** 22 May 2023

## Resumen

*Evaluación del polen de* Stryphnodendron polyphyllum *(Mart.) como potencial antioxidante y antibacteriano*

El polen de *Stryphnodendron polyphyllum*, conocido como barbatimão, es un problema para los apicultores con apiarios cerca de la planta, por la toxicidad para las abejas. El objetivo fue evaluar la actividad antioxidante, antibacteriana y determinar fenoles totales y taninos en el extracto etanólico. La actividad antioxidante se evaluó por método DPPH. La actividad antibacteriana por método de microdilución. Los taninos totales y fenoles se determinaron por método espectrofotométrico. Se identificó actividad inhibitoria y bactericida para *E. coli*, *P. aeruginosa*, *S. mutans*, *S. sobrinus*, *S. sanguinis*, *S. salivarius* y *L. paracasei*. Se determinó $EC_{50}$ de 7.42-mg/mL en la actividad antioxidante. Para compuestos fenólicos y tánicos, por comparación con curva de calibración, 3,12±0,005g/100g de muestra como ácido gálico y 7,3±0,03g/100g como ácido tánico. Se realizó cromatografía líquida de alta resolución para obtener el perfil cromatográfico.

**Palabras clave:** Actividad bactericida; Barbatimão; Apicultura; Polen, Fenoles totales; Taninos totales.

## Abstract

Pollen from *Stryphnodendron polyphyllum*, known as barbatimão, is a problem for beekeepers who have apiaries close to the plant, due to toxicity for bees. The objective was to evaluate the antioxidant, antibacterial activity and determine total phenols and tannins in ethanolic extract. Antioxidant activity was evaluated by DPPH method. Antibacterial activity was performed by microdilution method. Total tannins and phenols were determined by spectrophotometric method. We had results inhibitory and bactericidal activity for *E. coli*, *P. aeruginosa*, *S. mutans*, *S. sobrinus*, *S. sanguinis*, *S. salivarius* and *L. paracasei*. $EC_{50}$ of 7.42mg/mL was determined in the antioxidant activity. For phenolic and tannic compounds, the results, by comparison of calibration curve, were 3.12±0.005g/100g expressed as gallic acid and 7.3±0.03g/100g expressed as tannic acid. High performance liquid chromatography was performed to obtain the chromatographic profile.

**Key words:** Bactericidal activity; Barbatimão; Beekeeping; Pollen, Total phenols; Total tannins.

## Introduction

Medicinal plants are part of human evolution and part of the first therapeutic resources used by humanity. The use of herbal and medicinal plants is still common by the population, mainly due to the side effects caused by synthetic drugs (Monteiro & Brandelli 2017, Badke *et al.* 2019). About 25,000 plant species are used worldwide for the production of medicines from natural products and also marketed as herbal medicines. Currently, 85 species represent 236 herbal medicine formulations (Brasil 2021). Brazil has many species and great acceptance of the use of medicinal plants among the population, which generates revenue of approximately US$ 160 million annually, it is a promising and rapidly expanding market (Rodriguez 2016, Sanchez 2018, Medeiros *et al.* 2019).

Among the phytotherapic plants, the *Stryphnodendron polyphyllum* Mart. species stands out, belonging to the Fabaceae family, popularly known as barbatimão or barba-de-timão, bark-da-virgindade and faveira, occurring in the Cerrado and Caatinga biomes, in the states of Paraná, Minas Gerais, Bahia, São Paulo, Mato Grosso, Mato Grosso do Sul, Goiás, Distrito Federal and Tocantins, according to information deposited in the National Botanical Database Reflora (Forzza 2012, Zappi *et al.* 2015, Reflora, 2022).

The barbatimão, is native to the Brazilian cerrado, has a length of 4 to 5 meters, has an arboreal size, tortuous and thick trunk and an elongated crown, has an inflorescence with a variable number of small flowers, arranged in dense spikes, axillary, measuring about 10 cm in length and pod-like fruits, containing several light brown and slightly flattened seeds, being commonly found in the north of Minas Gerais, Brazil (Lorenzi 2010, Meira *et al.* 2016).

The reproductive characteristics of the barbatimão are marked by the existence of hermaphrodite flowers in the same specimen and they produce small amounts of nectar, attracting insects such as bees as the main pollinators (Felfili *et al.* 1999).

The present species represents a genetic resource of wide economic and medicinal importance, several applications are derived from barbatimão. The stem bark is the main used part of the plant, presents major constituents with high phenolic or tannic contents, demonstrating antioxidant, anti-inflammatory and antimicrobial effects (Ministério da Saúde 2014, Souza *et al.* 2018).

Only a small amount of research evaluates antimicrobial effects on microorganisms related to oral diseases using hydroalcoholic extracts of barbatimão (Soares *et al.* 2008, Ferreira *et al.* 2013). When it comes to bacterial resistance, some of the most important microorganisms are the Gram-positive *Staphylococcus aureus* Rosenbach and *Enterococcus faecalis* (Andrewes & Horder) and the Gram-negative *Escherichia coli* (Migula) and *Pseudomonas aeruginosa* (Schroeter), these are found more frequently in studies related to herbal medicines (Alves *et al.* 2016).

Studies with different parts of the plant are found in the literature, such as derivatives of bark, stem bark, stems, inner bark, leaves, broad beans, roots, fruits and seeds. However, the greatest concentration of studies is directed to the bark of the species (Miranda 2010, Thomazi 2010, Ministério da Saúde 2014).

Barbatimão pollen has unfavorable toxic effects for beekeeping, due to the content of tannins in the composition, the material can cause damage to the development of bee larvae, concentrations and pollen constituents are dependent on some factors such as: botanical origin, nature of the soil, genetic property of the plant and climatic condition (Amâncio 2014). The toxic effects may be associated with tannic acid (Santos 2000, Mendes *et al.* 2019), also known as gallotanic acid or gallotannin (3,4-dihydroxy-5-((3,4,5-trihydroxybenzoyl)oxy)benzoate), is a typical hydrolysable tannin with molecular formula $C_{76}H_{52}O_{46}$, a complex substance composed of a central glucose molecule and ten gallic acid molecules linked by esterification, it is easily soluble in water or alcohol and is obtained from plant sources (Santos 2007).

Diseases that especially attack the brood can cause more damage to the hives. The Brazilian Creates Bagged Disease (CBD) is one of the most common diseases, and it does not have a virus as the cause of the disease (Teixeira *et al.* 2003, Lopes *et al.* 2004, Castagnino *et al.* 2011), according to researchers, the disease is caused by the pollen of the barbatimão plant (Teixeira *et al.* 2003, Lopes *et al.* 2004, Cintra *et al.* 2005).

Intoxication by barbatimão pollen occurs at the time of feeding bee larvae, which prevents their development and leads to the loss of the larva (Castagnino *et al.* 2011). Such toxicity found in

pollen is due to a toxin from the tannin group, tannic acid, a polyphenol, a non-nutritive compound due to molecular complexity, even in small concentrations it can cause the disease (Teixeira *et al.* 2003).

At the time of barbatimão flowering, Creates Bagged Disease represents a big problem for beekeepers due to losses in the swarm, therefore, the present study aimed to analyze the pollen from the barbatimão plant to add value and encourage investments in the rational use of pollen in order to maintain the sustainability of the plant and minimize the impact caused to beekeepers.

## Materials and methods

### Material extraction

Barbatimão pollen was collected in the rural region of the city of Bocaiúva, north of Minas Gerais, in November, when the plant blooms. The material was extracted from the natural environment with registration in the National System for the Management of Associated Traditional Genetic Heritage (SisGen) with number AAE86b9.

### Preparation of the hydroethanolic crude extract

The crude hydroethanolic pollen extract was obtained with the addition of 10 g of pollen in 75 mL of ethanol 70% v/v , heated in a water bath at 70°C for 30 minutes. After it was submitted to filtration and taken to an oven to dry the solvent, after the extract was weighed, the yield was calculated and stored in a refrigerator at 5°C (Carpes *et al.* 2008).

### Determination of total tannins

Total tannin contents were obtained by adapting the methodology of Pansera *et al.* (2003). Dilutions of the crude extract at concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL were performed in water. To a 2 mL aliquot of the sample solutions, 2 mL of Folin-Denis reagent was added. The resulting solution was stirred vigorously and allowed to stand for 3 minutes. Afterwards, 2 mL of aqueous sodium carbonate solution 8% was added to the mixture, stirred and allowed to stand for 2 hours. Then, the sample was centrifuged at 2000 rpm to remove suspended materials. In parallel, tannic acid dilutions at concentrations of 0.004; 0.01; 0.04; 0.1; and 0.2 g/mL in water were prepared to run the analytical calibration curve. Dilutions of samples and standards were analyzed in a spectrophotometer at a wavelength of 725 nm. The total tannin content in the extract was quantified through the calibration curve of the tannic acid standard and expressed as equivalent to tannic acid per gram of extract.

### Determination of total phenols and antioxidant activity

For the test, a 51 mg/mL solution of the crude extract in ethanol 70% v/v was used as an initial solution and different dilutions of it. The test was performed according to the adaptation of the methodology by Barth, *et al.* (2018), determined by spectrophotometry by the DPPH method, the results were compared to the gallic acid standard. A 20 µg/mL gallic acid solution was prepared and dilutions were performed to obtain the curve. The tests were performed in triplicate, the averages of the results were plotted in a graph of concentration versus absorbance, in parallel a control was performed using DPPH solution and ethanol 70%, the reading in the spectrophotometer was at a wavelength of 517 nm. The reduction of DPPH was observed through the absorbance and the results of total phenols were presented as equivalence of gallic acid through the equation derived from linear regression. The percentage of antioxidant activity (AA%) was obtained through the next equation and then expressed as $CE_{50}$, which is the efficiency of an antioxidant to reduce the initial concentration of DPPH by 50%:

$$\%AA = \left(1 - \frac{Abs_{Sample}}{Abs_{Control}}\right) * 100$$

%AA: *Percentage of Antioxidant Activity*
Abs Sample: *Sample absorbance*
Abs Control: *Control absorbance*

### Antibacterial activity

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of the antimicrobial agent capable of inhibiting bacterial growth. To determine the MIC of the aerobic and microaerophilic bacteria included in the study, the microdilution method recommended by the CLSI (2012) was performed, with some modifications.

Samples were dissolved in dimethylsulfoxide (DMSO) and diluted with Brain Heart Infusion broth. Then, twelve concentrations of isolated compounds ranging from 0.9765 to 2000 µg/mL

were tested in a 96-well microplate.

The bacteria used in this study were obtained from the American Type Culture Collection (ATCC), aerobic bacteria: *S. aureus* (ATCC BAA44), *S. aureus* (ATCC 6538), *P. aeruginosa* (ATCC 29853), *E. coli* (ATCC BAA198), E. coli (ATCC 25922), *Streptococcus salivarius* (Orla-Jensen) (ATCC 25975) and *E. faecalis* (ATCC 4082); microaerophilic bacteria: *Streptococcus mutans* Clarke (ATCC 25175), *Streptococcus sobrinus* Coykendall (ATCC 33478), *Streptococcus sanguinis* White & Niven (ATCC 10556), *S. salivarius* (ATCC 25975) and *Lactobacillus paracasei* Collins *et al.* (ATCC 11578). Inoculums were adjusted to a cell concentration of 5 x 10 5 CFU/mL.

The plates containing the aerobic bacteria and the plates containing the microaerophilic bacteria were incubated at 37 ºC for 24 hours in (10% $CO_2$ - flask with candle). After incubation, 30 µL of 0.02% aqueous resazurin (7-hydroxy-3H-phenoxazin-3-one) solution was added to each well to reveal bacterial growth (Sarker *et al.* 2007). DMSO 5% (v/v) was used as a negative control. As a positive control, tetracycline and vancomycin (0.0115 to 5.9 µg/mL) and chlorhexidine (0.115 to 59.0 µg/mL) were used. Inoculum was included to monitor bacterial growth. To determine the Minimum Bactericidal Concentration (MBC), defined as the lowest concentration of the test sample without any bacterial growth, a 10 µL aliquot of the inoculum was removed from each well prior to the addition of resazurin and plated on Brain Heart Infusion Agar plates, being observed absence/presence of bacterial growth after incubation for 24 hours at 37 ºC. The experiments were performed in triplicate in three different experiments.

### Chromatographic profile

The investigation of the chromatographic profile was carried out using high performance liquid chromatography. The equipment used was the Waters liquid chromatograph, equipped with a model 1525 binary pump, model 717 automatic injector, model 2996 photodiode array detector and Empower Pro software. A C18 column, 250 mm × 4.6 mm and 5 µm particle Spherisorb, Waters, was used in the separation of compounds. The mobile phase used was a mixture of methanol:water (70:20) according to Pellenz *et al.* (2018), being pumped in isocratic mode, and the reading was performed at a wavelength of 220 nm.

## Results and discussion

### Determination of total tannins

The result obtained in the test is showed in Table 1. The linearity coefficient for the determination of the tannic acid calibration standard curve was 0.9928, and a tannic acid equivalence of 7.30 ± 0.030g/100 g of sample, expressed as total tannins, could be quantified. Tannins are found in almost all plants throughout the world, however concentrations vary with significant proportions in some plants, usually found in large amounts in tree bark (Ashok & Upadhyaya 2012). According to Brasil (2019), the bark of the barbatimão plant has at least 8% of total tannins. Other references report concentrations of approximately 20% of total tannins found in the plant bark (Audi *et al.* 2004, Lopes *et al.* 2009, ANVISA 2022). In the present work, approximately 7.3% of total tannins present in the pollen extract were quantified, a result similar to that reported by Santos (2000), who quantified the total tannins of pollen and inflorescence of the barbatimão, finding 8 and 21% respectively.

| Pollen extract (100g) | | |
|---|---|---|
| | Equivalence | Deviation |
| Tannic Acid | 7.30 g | 0.030 |
| Gallic Acid | 3.12 g | 0.005 |
| **$CE_{50}$** | 7.42 mg/mL | - |

**Tabla 1.** Equivalencia de ácido tánico y ácido gálico del extracto de polen.

**Table 1.** Equivalence of tannic and gallic acid from pollen extract.

The quantification of the total tannins was evaluated comparing with the standard of tannic acid because it has a high concentration in the extract as reported by several authors (Lopes *et al.* 2009, Ricardo & Brandão 2018, ANVISA 2022). The presence and high concentration of tannins found in the extract, according to Silva *et al.* (2010) add to this, antimicrobial and anti-inflammatory properties.

### Total phenols and antioxidant activity

The result of 0.9948 of the linearity coefficient of the calibration curve of the gallic acid standard was expressed in relation to absorbance and concentrations. A gallic acid equivalence of 3.12 ±

0.005 g/100 g of sample can be quantified, expressed as total phenols. For the antioxidant activity, an EC50 value of 7.42 mg/mL was obtained (Table 1). The phenolic compounds of plants have received attention because they fit into several categories such as simple phenols, phenolic acids, flavonoids, tannins and lignins, which stand out for their reducing capacity acting in the neutralization of the oxidative process (Santos 2007). The amount of phenols found in the extract of the present study was approximately 3.12 g of gallic acid for every 100 g of extract. The literature presents phenol content in the bark, leaves and roots of different plants from the Brazilian Cerrado, finding results between 70.6 and 20.5 g of gallic acid for every 100 g of extract (Sousa *et al.* 2007). For barbatimão bark extract 15.8 g of gallic acid for every 100 g of extract (Silva 2018). The high concentrations found by the authors are justified by the fact that they are quantified in plant barks, where the concentration of phenols is higher (ANVISA 2022). Also, the higher the content of phenolic compounds, the greater the antioxidant capacity. The result of the antioxidant activity of the pollen extract made it possible to calculate the $CE_{50}$ through the straight line equation (Fig. 1). $CE_{50}$ is the ability of the antioxidant to reduce the initial concentration of DPPH present in the solution by 50%.

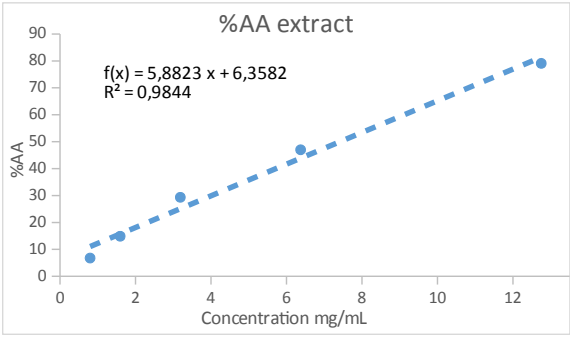


**Figura 1.** Extracto de actividad antioxidante.
**Figure 1.** Extract antioxidant activity.

The pollen extract concentration capable of reducing the DPPH solution by 50% was 7.42 mg/mL, a result found using a low extract concentration. It is also worth mentioning that a slightly higher concentration of 12.75 mg/mL of the same extract was effective to reduce DPPH by approximately 80%. On the other hand, results pointed out by researchers showed that the antioxidant activity for barbatimão bark is found with an CE50 of approximately 10 µg/mL (Farias *et al.* 2013, Baldivia *et al.* 2018, Silva 2018). This comparison is possibly expected since the plant pollen has lower concentrations of flavonoids as reported in the present work.

## Antibacterial activity

When evaluating the in vitro antimicrobial activity of the crude extract (Table 2), positive results can be observed in the inhibition of different bacteria used in the study. The action of barbatimão bark against bacteria has already been reported and shows positive results against different types of bacteria, and is due to the richness in tannins, flavonoids and the set of substances present in the plant (Soares *et al.* 2008, Da Costa *et al.* 2020, Santos *et al.* 2021).

Most oral bacteria were used in the present study, and the results of MIC and MBC against bacteria are mostly favorable. Because the chromatographic profile of the extract has similarity to the flavonoid rutin, the antimicrobial activity of rutin was also evaluated in the investigation (as shown in Table 2), the similarity can also be noted in the results compared to the concentrations of MIC and MBC in most of the cases. Chemical structures of flavonoids and phenolics found in extracts of some plants may demonstrate significant antimicrobial activity (Mahmood *et al.*

| | Barbatimão Extract | | Rutin | |
|---|---|---|---|---|
| | MIC | MBC | MIC | MBC |
| *Escherichia coli* ATCC 25922 | 2000 | 2000 | 2000 | 2000 |
| *Escherichia coli* ATCC BAA 198 | 2000 | 2000 | 2000 | 2000 |
| *Pseudomonas aeruginosa* ATCC 29853 | 1000 | 2000 | 2000 | 2000 |
| *Staphylococcus aureus* ATCC 6538 | >2000 | >2000 | >2000 | >2000 |
| *Staphylococcus aureus* ATCC BAA 44 | >2000 | >2000 | >2000 | >2000 |
| *Streptococcus mutans* ATCC 25175 | 2000 | 2000 | 2000 | >2000 |
| *Streptococcus sobrinus* ATCC 33478 | 2000 | 2000 | 2000 | 2000 |
| *Streptococcus sanguinis* ATCC 10556 | 500 | 1000 | 1000 | 1000 |
| *Streptococcus salivarius* ATCC 25975 | 2000 | 2000 | 2000 | 2000 |
| *Lactobacillus paracasei* ATCC 11578 | 2000 | 2000 | 2000 | 2000 |
| *Eenterococcus faecalis* ATCC 4082 | 2000 | >2000 | 2000 | >2000 |

**Tabla 2.** MIC y MBC de extracto de barbatimão y rutina (µg/mL).
**Table 2.** MIC and MBC of barbatimão extract and (µg/mL). (µg/mL).

2021). For the bacterium *S. sanguinis* the MIC and MBC were only 500 and 1000 ug/mL respectively, for all other bacteria studied the MIC and MBC were approximately 2000 ug/mL. The MIC of the ethanol extracts in different parts of the *Moringa oleifera* Lam. plant showed concentrations of 50 mg/mL of the ethanol extract for *P. aeruginosa*, and CBM was not reported in the study (Prabakaran *et al.* 2018), with the concentration of the plant extract being about 25 times higher than that used in the present study. The MIC and MBC in ethanol extract of Eugenia involucrata DC leaves for the bacteriae *P. aeruginosa*, *S. aureus*, *E. coli* and *E. faecalis* were 12.5 and 12.5, 3.12 and 6.25, 12.5 and 25, 12.5 and 50 mg/mL, respectively (Toledo *et al.* 2021), the author reports excellent antimicrobial activity for the bacteria studied, and for most of the bacteria studied the concentration of the plant extract was about 10 times higher than the concentration used in the present study. On the other hand, MIC and MBC of barbatimão ethanol extract were not observed within the concentrations studied for the S. aureus bacterium, and MBC for the *E. faecalis*.

The MIC of barbatimão bark against oral bacteriae *E. faecalis*, *S. salivarius*, *S. mutans*, *S. sanguinis*, *S. sobrinus* and *L. paracasei* was reported in the literature, showing promising results with concentrations close to 500 ug/mL of the plant (Soares *et al.* 2008). Values relatively close to those found in the present work were reported, however, in the present work plant pollen was used, generating results that demonstrate great antimicrobial activity of the barbatimão pollen extract.

The extract results presented were not directly compared with the positive controls (Table 3), since the positive controls are pure substances and the extract is a complex mixture. The positive control was used only for comparison with the conventional treatment.

*S. sanguinis* is one of the first bacteria to adhere to tooth enamel, it favors the colonization of the tooth surface by other bacteria (Buischi 2000), in which the plant extract obtained greater antimicrobial activity, thus, the results demonstrate a proposal for the development of products capable of reducing or inhibiting the growth of the bacteria studied, preventing dental caries. The use of the crude plant extract could offer the removal of the actives purification process, reducing costs and enabling the possible use of the extract as a phytotherapic.

## Chromatographic profile

The chromatographic profile evaluated can be observed (Fig. 2), and it was evaluated against the chromatographic profile of rutin, demonstrating significant similarity to this flavonoid. The chromatographic profile of the extract was collected with good resolution and evaluated against the rutin standard (Fig. 2). Similarity of peaks was observed between 2.5 and 3.5 minutes, confirming the presence of flavonoids with the chemical skeleton similar to rutin. The chromatographic profile obtained is similar to that found in the literature, according to a study carried out by Sabino (2018), where the chromatographic profile of the barbatimão leaf showed the two highest intensity peaks, described as polyphenols,gallic acid and

| **Positive control results in µg/mL** | | | | | | |
|---|---|---|---|---|---|---|
| | Tetraciclin | | Chlorhexidine | | Vancomicin | |
| | MIC | MBC | MIC | MBC | MIC | MBC |
| ***Escherichia coli* ATCC25922** | 0.3688 | 0.3688 | - | - | - | - |
| ***Escherichia coli* ATCC BAA 198** | 0.7375 | 0.7375 | - | - | - | - |
| ***Pseudomonas aeruginosa* ATCC 29853** | 5.9 | 5.9 | - | - | - | - |
| ***Staphylococcus aureus* ATCC 6538** | 0.0922 | 0.0922 | - | - | - | - |
| ***Staphylococcus aureus* ATCC BAA 44** | - | - | - | - | 0.7375 | 0.7375 |
| ***Streptococcus mutans* ATCC 25175** | - | - | 0.922 | 0.922 | - | - |
| ***Streptococcus sobrinus* ATCC 33478** | - | - | 3,.88 | 3.688 | - | - |
| ***Sstreptococcus sanguinis* ATCC 10556** | - | - | 1.844 | 1.844 | - | - |
| ***Streptococcus salivarius* ATCC 25975** | - | - | 1.844 | 1.844 | - | - |
| ***Lactobacillus paracasei* ATCC 11578** | - | - | 14.75 | 14.75 | - | - |
| ***Eenterococcus faecalis* ATCC 4082** | - | - | 1.844 | 1.844 | - | - |

**Tabla 3.** MIC y MBC de los controles positivos. (-) no realizado.

**Table 3.** MIC and MBC of the positive controls. (-) unrealized.

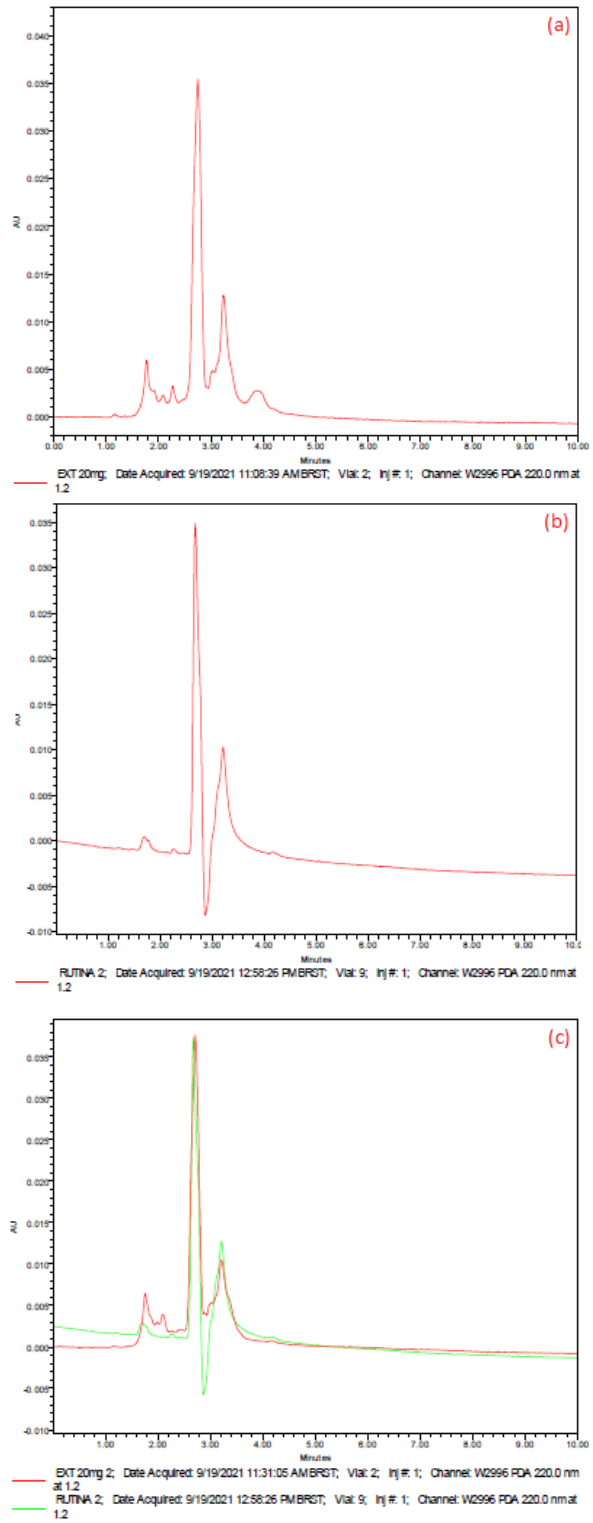


**Figura 2.** Perfil cromatográfico. **A:** extracto de barbatimão; **B:** rutina; **C:** extracto de barbatimão contra la rutina.

**Figure 2.** Chromatographic profile. **A:** barbatimão extract; **B:** rutin; **C:** barbatimão extract against rutin.

proanthocyanidin, respectively. As can be seen (Fig.2), image (a), the separation of the compounds present in the extract can be easily observed, on the other hand, it was not complete, resulting in approximately 1.5% of rutin present in the extract. Due to the non-complete separation of the peaks, the result obtained via chromatography was expressed as approximate.

## Conclusion

The results calculated in this research show that the barbatimão pollen extract has significant amounts of phenolic and tannic compounds, which may be responsible for the remarkable antibacterial and antioxidant action. These are the main findings of this study, which will serve to encourage development research for products derived from plant pollen, as few studies directed to barbatimão pollen are available in the literature.

## Acknowledgments

To the State University of Montes Claros (UNIMONTES) and Federal University of Uberlândia (UFU) for the laboratory facilities and supplies. The Coordination for the Improvement of Higher Education Personnel (CAPES).

## References

Alves JE, Souza TA, Lacerda GA & Prince PMA. 2016. Atividade antibacteriana do extrato hidroalcoólico de jatobá-do-cerrado (Hymenaea stigonocarpa Mart. Ex Hayne) e barbatimão (Stryphnodendron adstringens (Mart.) (Coville). Revista Bionorte 5(1): 32-45.

Amâncio DCPG. 2014. Compostos bioativos do pólen. Coimbra: Faculdade de Farmácia da Universidade de Coimbra, Tese de Doutorado.

ANVISA. 2022. Farmacopeia Brasileira, 6nd ed. Agência Nacional de Vigilância Sanitária, vol. 1. Available in https://www.gov.br/anvisa/pt-br/assuntos/farmacopeia/farmacopeia-brasileira (accessed on 1-VI-2022).

Ashok PK & Upadhyaya K. 2012. Tannins are astringent. Journal of pharmacognosy and phytochemistry 1(3): 45-50.

Audi EA, Toledo CEM, Santos FS, Bellanda PR, Alves WP, Ueda TN, . . . Mello JCP. 2004. Biological activity and quality control of extract and stembark from Stryphnodendron adstringens. Acta Farmaceutica Bonaerense 23(3): 328-33.

Badke MR, Cogo SB, Ilha AG, Heisler EV, Schimith MD & Sacramento HT. 2019. Panorama brasileiro dos serviços de plantas medicinais e fitoterápicos. Revista de Enfermagem da UFSM 9: 1-19. https://doi.org/10.5902/2179769233655

Baldivia DS, Leite DF, Castro DTH, Campos JF, Santos UP, Gamero EJP, . . . Santos EL. 2018. Evaluation of in vitro antioxidante and anticâncer properties of the aqueous extract from the stembark of Stryphno-

dendron adstringens. International Journal of Molecular Sciences. vol. 19, no. 8, 1908-2432. https://doi.org/10.3390/ijms19082432.

Barth EF, Pinto LS, Dileli P, Biavatti DC, Silva YL, Bortolucci W, . . . Laverde AJ. 2018. Biological screening of extracts from leaf and stembark of Crotonfloribundus Spreng. (Euphorbiaceae). Brazilian Journal of Biology 78(4): 601-608. https://doi.org/10.1590/1519-6984166522

Brasil MS. 2021. Formulário de Fitoterápicos da Farmacopeia Brasileira, 2nd ed. Agência Nacional de Vigilância Sanitária: Anvisa.

Buischi YP. 2000. Promoção de saúde bucal na clínica odontológica. São Paulo: Artes Médicas.

Carpes ST, Prado A, Moreno IAM, Mourão GB, Alencar SM & Masson ML. 2008. Avaliação do potencial antioxidante do pólen apícola produzido na região Sul do Brasil. Química Nova 31(7): 1660-1664. https://doi.org/10.1590/S0100-40422008000700011.

Castagnino GLB, Message D & Marco PJ. 2011. Fornecimento de substituto de pólen na redução da morte de Apis melífera L. causada pela Cria Ensacada Brasileira. Ciência Rural 41(10): 1838-1843. https://doi.org/10.1590/s0103-84782011001000027.

Cintra P, Malaspina O & Bueno OC. 2005. Plantas tóxicas para abelhas. Arquivos do Instituto Biológico 72(4): 547-551.

CLSI. 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard, 9th ed., CLSI document M07-A9. Wayne, Pennsylvania: Clinical and Laboratory Standards Institute.

Costa CES, Andrade RS, Almeida RSC, Moura SK, Maciel SM & Frederico RCP. 2020. Antimicrobial Activity of Barbatimão Glycol Extracton Streptococcus mutans. Journal of Health Sciences 22(2): 131-134. https://doi.org/10.17921/2447-8938.

Farias KS, Santos TSN, Paiva MRAB, Almeida SML, Guedes PT, Vianna ACA, . . . Bueno NR. 2013. Antioxidant properties of species from the Brazilian cerrado by diferente assays. Revista Brasileira de Plantas Medicinais 15(4): 520-528. https://doi.org/10.1590/S1516-05722013000400008.

Felfili JM, Silva MCJ, Dias BJ & Rezende AV. 1999. Estudo fenológico de Stryphnodendron adstringens (Mart.) (Coville) no cerrado sensu stricto da Fazenda Água Limpa no Distrito Federal, Brasil. Revista Brasileira de Botânica 22(1): 1–10. https://doi.org/10.1590/S0100-84041999000100011

Ferreira EC, Silva JLL & Souza RF. 2013. As propriedades medicinais e bioquímicas da planta Stryphnodendron adstringens “barbatimão”. Biológicas & Saúde 3((11): 14-32. https://doi.org/10.25242/886831120139

Forzza RC, Baumgratz JFA, Bicudo CEM, Canhos DAL, Carvalho AA, Coelho MAN, . . . Leitman PM. 2012. New Brazilian Floristic List Highlights Conservation Challenges. BioScience 62(1): 39-45. https://doi.org/10.1525/bio.2012.62.1.8

Lopes GC, Sanches ACC, Toledo CEM, Isler AC & De Mello JCP. 2009. Determinação quantitativa de taninos em três espécies de Stryphnodendron por cromatografia líquida de alta eficiência. Brazilian Journal of Pharmaceutical Sciences 45(1): 135-143. https://doi.org/10.1590/s1984-82502009000100017.

Lopes MTR, Gonçalves J, Preira FDM & Camargo RCR. 2004. Doenças e inimigos naturais das abelhas. Embrapa: Meio-Norte-Documentos.

Lorenzi H. 2010. Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil. Vol. 2. São Paulo: Instituto Plantarum.

Mahmood MS, Ashraf A, Ali S, Siddique AB, Asad F, Abbas RZ, . . . Rafique A. 2021. Portrayal of Punica granatum L. peel extract through High Performance Liquid Chromatography and antimicrobial activity evaluation. Brazilian Journal of Biology 83: [7]. https://doi.org/10.1590/1519-6984.244435.

Medeiros FS, Sá GB, Dantas MKL & Almeida MGVM. 2019. Plantas medicinais comercializadas na feira livre do município de Patos, Paraíba. Revista Verde de Agroecologia e Desenvolvimento Sustentável 14(1): 150-155. https://doi.org/10.18378/rvads.v14il.5448

Meira MR, Cabacinha CD, Gama AT, Martins ERM & Figueiredo LS. 2016. Caracterização estrutural do barbatimão (Stryphnodendron adstringens (Mart.) (Coville) no cerrado do norte de Minas Gerais. Ciência Florestal 26(2): 627-638. https://doi.org/10.5902/1980509822762

Mendes VM, Portes KDP, & Zaluski R. 2019. Barbatimão Stryphnodendron spp.: um problema para a apicultura. Presented at the 2019 XII Mostra Científica Famez & I Mostra Regional de Ciências Agrárias, Campo Grande, MS.

Ministério da Saúde. 2014. Monografia da espécie Stryphnodendron adstringens (Mart.) (Coville) (Barbatimão). Ministério da Saúde e Anvisa.

Miranda MA. 2010. Atividade antimicrobiana das soluções de Barbatimão, Mamona e Clorexidina utilizadas na Endodontia. Avaliação comparativa in vitro. Ribeirão Preto: Universidade de São Paulo, Dissertação de Mestrado em Odontologia.

Monteiro DCS & Brandelli CLC. 2017. Farmacobotânica: Aspectos Teóricos e Aplicação. Porto Alegre: Artmed Editora.

Pansera MR, Santos ACA, Paese K, Wasum R, Rossato M & Rota LD, . . . Serafini LA. 2003. Análise de taninos totais em plantas aromáticas e medicinais cultivadas no Nordeste do Rio Grande do Sul. Revista Brasileira de Farmacognosia 13(1): 17-22. https://doi.org/10.1590/S0102-695X2003000100002.

Pellenz NL, Barbisan F, Azzolin VF, Duarte T, Bolignon A &, Mastella MH, . . . Duarte MMMF. 2018. Analysis of in vitro cyto- and genotoxicity of Barbatimão extract on human keratinocytes and fibroblasts. BioMed Research International 2018: ID 1942451 [11]. https://doi.org/10.1155/2018/1942451.

Prabakaran M, Kim SH, Sasireka A, Chandrasekanan M & Chung M. 2018. Polyphenol composition and antimicrobial activity of various solvent extracts from different plant parts of Moringa oleifera. Food Bioscience 26: 23-29. https://doi.org/10.1016/j.fbio.2018.09.003.

Reflora, 2022. Herbario Virtual Reflora. Available in

https://reflora.jbrj.gov.br/reflora/herbarioVirtual/ConsultaPublicoHVUC/ConsultaPublicoHVUC.do (Accessed on 1-VII-2022)

Ricardo LM & Brandão MGL. 2018. Stryphnodendron adstringens (Mart.) (Coville). in Medicinal and Aromatic Plants of South America (Máthé A, ed.). Dordrecht: Springer, pp. 431-437.

Rodrigues W. 2016. Competitividade e mudança institucional na cadeia produtiva de plantas medicinais no Brasil. Interações (Campo Grande) 17(2): 267-277. https://doi.org/10.20435/1984042X2016210

Sabino APL, Eustáquio LMS, Miranda ACF, Biojone C, Mariosa TN & Gouvêa CMCP. 2018. Stryphnodendron adstringens ("Barbatimão") leaffraction: chemical characterization, antioxidant activity, and cytotoxicity towards human breast cancer cell lines. Applied biochemistry and biotechnology 184(4): 1375-1389. https://doi.org/10.1007/s12010-017-2632-z.

Sanchez NA. 2018. As plantas medicinais brasileiras na literatura médica portuguesa: análise do Código Pharmaceutico Lusitano e possíveis desdobramentos. São Paulo: Pontífica Universidade Católica de São Paulo. Dissertação de Mestrado em História da Ciância.

Santos ES. 2007. Microrganismos promissores para a degradação de compostos fenólicos presentes em bagaço-de-cana, lodo e águas residuárias de agroindústria sucro-alcooleira. Alagoas: Universidade Federal de Alagoas, Dissertação de Mestrado em Biotecnologia.

Santos JPCL, Santos ICM, Andrade TI, Nascimento TCES, Falcão REA, Moreira KA & Nascimento PLA. 2021. Potencial antimicrobiano do extrato etanólico da casca de Stryphnodendron barbatimam (Mart.) ante microrganismos de interesse médico-odontológico. RSBO. vol. 18, no. 1, 23-30.

Santos MLA. 2000. Análise dos constituintes químicos do pólen e da inflorescência de Stryphnodendron polyphyllum, em relação à cria-ensacada brasileira em Apis mellifera. Viçosa: Universidade Federal de Viçosa, Dissertação de Mestrado em Ciências.

Sarker SD, Nahar L & Kumarasamy Y. 2007. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods 42(4): 321-324. https://doi.org/10.1016/j.ymeth.2007.01.006.

Silva LAF, Silva JA, Lima CRO & Dambros CE, Cardoso VS. 2010. Uso popular do barbatimão. In Manual do Barbatimão (Silva LAF, Eurides D, Paula JR, Lima CRO & Moura MI, eds.). Kelps: Goiânia, pp.79-85.

Silva NF. 2018. Composição química, efeitos antifúngico, antioxidante e citotóxico de cascas de Stryphnodendron adstringens (Mart.) (Coville) (Barbatimão). Recife: Universidade Federal de Pernambuco, Dissertação de Mestrado em Morfotecnologia.

Soares SP, Vinholis AHC, Casemiro LA, Silva MLA, Cunha WR & Martins CHG. 2008. Antibacterial activity of the crude hydroalcoholic extract of Stryphnodendron adstringens on dental caries microorganisms. Revista Odonto Ciência 23(2): 141-144.

Sousa CMM, Silva HR, Vieira GM, Ayres MCC, Costa CLS, Araújo DS, . . . Chaves MH. 2007. Fenóis totais e atividade antioxidante de cinco plantas medicinais. Química nova 30(2): 351-355. https://doi.org/10.1590/S0100-40422007000200021.

Teixeira EW, Serrão JE & Oliveira JA. 2003. Ácido tânico causa danos ao epitélio ventricular de larvas da abelha Apis melífera L. (Hymenóptera: apidae). Boletim de Indústria Animal 60(2): 155-161.

Thomazi GOC. 2010. Investigação da atividade antibacteriana de espécies de plantas do Cerrado contra bactérias responsáveis por infecções do trato urinário. Palmas: Universidade Federal do Tocantins, Dissertação de Mestrado em Ciências do Ambiente.

Toledo AG, Souza JGL, Santana CB, Mallmann AP, Santos CV, Corrêa JM & Pinto FGS. 2021. Antimicrobiana, atividade antioxidante e prospecção fitoquímica de Eugenia involucrata DC. extratos de folhas. Brazilian Journal of Biology 83: [9] https://doi.org/10.1590/1519-6984.245753.

Zappi DC, Filardi FLR, Leitman P, Souza VC, Walter BMT, Pirani JR, . . . Forzza RC. 2015. Growing knowledge: an overview of Seed Plant diversity in Brazil. Rodriguésia 66(4): 1085–1113. https://doi.org/10.1590/2175-7860201566411