Bergenin of *Peltophorum dubium* (Fabaceae) Roots and Its Bioactive Semi-Synthetic Derivatives

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This work describes the isolation of pure bergenin from *Peltophorum dubium* roots with good yields and its further derivatization through Williamson synthesis. The alkyl derivatives of the bergenin were identified by nuclear magnetic resonance and mass spectrometry data analysis. Among them, three derivatives were reported for the first time herein, 8,10-dihexyl-bergenin, 8,10-didecyl-bergenin, and 8,10-ditetradecyl-bergenin, along with three previously reported ones (8,10-dimethylbergenin, 8-methylbergenin, 8,10-dioctyl-bergenin). Most derivatives of the bergenin displayed moderate cytotoxicity against *Artemia salina*, except the 8,10-dihexyl-bergenin (lethal concentration doses for 50% (LC\(_{50}\)) = 70.55 µg mL\(^{-1}\)). The antimicrobial assay showed that all derivatives selectively inhibited the Gram-positive bacteria *Staphylococcus aureus*, and two of them (8,10-dihexyl-bergenin and 8,10-didecyl-bergenin) had promising activities (minimum inhibitory concentration (MIC) = 5.1-6.2 µmol L\(^{-1}\)). In addition, bergenin displayed interesting inhibition on acetylcholinesterase (half maximal inhibitory concentration (IC\(_{50}\)) = 141.19 ± 0.41 µmol L\(^{-1}\)), while its semi-synthetic derivatives displayed modest activity. Our results revealed that the antibacterial activity of bergenin could be greatly improved through its structural modification.

**Keywords:** bergenin, alkyl derivatives, antimicrobial activity, *Peltophorum dubium*

**Introduction**

Nature is an extremely rich source of highly diverse and innovative chemical structures. Although modern strategies such as combinatorial chemistry and molecular modeling have played an important role in drug discovery, the literature has reported that the prospection of natural products continues being a successful point of start to drug innovation.\(^1\) On the other hand, some natural products do not have adequate potency, selectivity, and pharmacokinetic properties to enter into clinical studies. The planning of chemical changes in the structures of natural products may circumvent the low yields from classical phytochemistry and provide derivatives that will be true lead compounds for pharmaceutical industries.\(^2\)

*Peltophorum* genus is geographically distributed across several regions of South America, Mexico, Southern United States, Africa, Southeast Asia, and Northern Australia, with some species found only in paleotropics.\(^3\) It belongs to the Caesalpinioideae subfamily of the Leguminosae (Fabaceae) and, to date, seven species are known (*Peltophorum dubium*, *P. pterocarpum*, *P. africanum*, *P. inermi*, *P. ferrugineum*, *P. dasyates* and *P. vogelianum*). Some of these plants are used as folk medicines, such as the roots, leaves, and barks of *P. africanum* that are employed to treat infections and chronic diseases, such as arthritis.\(^4\) However, the chemical composition of plants of this genus is still scarce. There are reports about the presence of flavonoid, flavonoid glycosides, triterpenes, gallic acid, and its C-glycoside derivatives, such as bergenin (1) and nor-bergenin in this genus species.\(^5\)

*Peltophorum dubium* (Spreng.) Taub. is a tree belonging to the Fabaceae family and grows in South American countries.\(^6\) This tree is easy to adapt to tropical habitats, and it has economic and ornamental importance. Its wood is used in civil buildings, in furniture and naval industries.\(^7\)

Bergenin (1) is a C-glycoside of 4-O-methylgallic acid that exhibits wide biological activities such as anti-inflammatory,\(^8\) antinociceptive,\(^9\) hypolipidemic,\(^10\) anti-HIV,\(^11\) hepatoprotection,\(^12\) neuroprotective,\(^13\) among others. Owing to such a broad spectrum of bioactivities associated with bergenin (1), studies have been devoted to obtaining more
active chemical derivatives. Bergenin (1) contains five hydroxyl groups that may be esterified with a variety of fatty acids. Within this context, some derivatives of 1 displayed enhanced antioxidant activity, and the preparation of lipophilic derivatives of the bergenin (1) could lead to new compounds with interesting anti-inflammatory activities.

Previously, the antimicrobial activity of crude extracts of 39 plant species from Argentina has been reported. The methanolic extract of *P. dubium* displayed interesting inhibitory activity against *Staphylococcus aureus*. This seminal study indicates that this extract should be further investigated for the possible presence of antibacterial compounds. In addition, the acetylcholinesterase (AChE) inhibitory activity of bergenin (1) was recently reported.

As part of our ongoing investigations on bioactivities of derivatives of natural products, this study describes a simple procedure for the isolation of bergenin (1) in good yields from roots of *P. dubium*. In sequence, 1 was transformed into alkyl derivates through a semi-synthetic approach. The biological potential of bergenin (1), along with its derivatives, was accessed through *in vitro* antibacterial and inhibition of AChE assays.

**Experimental**

**General experimental procedures**

Melting point was determined by a digital Mikroquimica equipment. Optical rotation was measured at 20 ºC in a PerkinElmer 343 Polarimeter at 589 nm. Infrared spectrum (IR) was acquired on a Shimadzu model IRPrestige-21 FTIR spectrophotometer in KBr film. Nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz with a Bruker Avance III spectrometer. Residual ¹H resonance from deuterated methanol (Cambridge isotope Lab., Andover, MA, USA) peak at δH 3.31 was used to reference the ¹H NMR spectra with the methyl resonance of tetramethylsilane (TMS) at 0.0 ppm. Electrospray ionization mass spectroscopy (LR-ESIMS) spectra were recorded with a Bruker mass spectrometer model Amazon Speed ETD operating in the positive ion mode with the following conditions: capillary voltage of 4500 V, dry gas flow of 5 L min⁻¹, and nitrogen as the nebulizer gas was used. Cholinesterase inhibitory activity was determined using a 96-well microplate reader Stat Fax-2600.

**Plant material**

Roots of *P. dubium* (Spreng.) Taub. were collected in the Ondina campus of Federal University of Bahia (13°00'19.7"S 38°30'37.9"W), Salvador, Bahia, Brazil in March of 2017. The biological material was identified by Prof Maria L. S. Guedes. A voucher specimen was deposited in the Alexandre Leal Costa herbarium, under voucher code R196538. The access to the specimen was registered in the Sistema Nacional de Gestão do Patrimônio Genético e Conhecimento Tradicional Associado (SisGen) under code A56BAD6.

**Extraction and purification of the bergenin**

The roots (1551.14 g) were dried in a forced circulating oven (40 ºC) during 48 h, powdered and submitted twice to maceration in 4 L of methanol (MeOH, Anidrol PA, São Paulo, Brazil) for 48 h. After vacuum evaporation of the solvent, the MeOH extract (37.19 g) was partitioned sequentially between 400 mL of MeOH:H₂O (8:2) and 3 x 60 mL of hexane (14.7 g), CHCl₃ (8.9 g), and 9.2 g of ethyl acetate (EtOAc, Anidrol, São Paulo, Brazil). The CHCl₃ soluble fraction was submitted to a chromatographic column (CC) containing silica gel 60 (Aldrich, St. Louis, USA), and it was eluted with CH₂Cl₂:MeOH (8:2). The fifth fraction (50 mL) furnished the pure bergenin (1.29 g, 0.379% of yield).

**Synthesis of derivatives of the bergenin (2-6)**

Methyl iodine (Sigma-Aldrich, St. Louis, USA), 1-bromo-hexane (C₆H₁₃Br), 1-bromo-octane (C₈H₁₇Br), 1-bromodecane (C₁₀H₂₁Br), and 1-bromotetradecane (C₁₄H₂₉Br) were employed for the synthesis of derivatives of the bergenin (2-6). For this, a solution composed of bergenin (0.9-1 mmol), CaCO₃ (4-5 mmol, Synth, Diadema, Brazil), and N,N-dimethylformamide (DMF) (10 mL, Merck, Darmstadt, Germany) was stirred for 30 min at 80 ºC. Sequentially, the alkyl halides (4-5 mmol) were carefully added drop by drop to the mixture, and the reactional system was kept stirring for 6-8 h at 80 ºC. The reactions were monitored by thin layer chromatography (TLC, Aldrich, St. Louis, USA) eluted with CH₂Cl₂:MeOH 8:2 and the chromatographic plates were visualized under UV light. The reaction was finished when bergenin was not detected in the TLC plates anymore. Thus, the reactional solution was partitioned employing CH₂Cl₂ and the organic layer was rinsed successively with HCl (10 mol L⁻¹) and NaHCO₃ (5%) aqueous, added Na₂SO₄, filtered and the solvent submitted to vacuum evaporation. The solid was purified by CC in silica gel eluted with CH₂Cl₂:MeOH 8:2 in order to obtain the pure derivatives (Scheme 1).

The same reaction methodology was used to obtain all the derivatives of the bergenin (2-6), varying only the reagents as described above. This methodology is a
nucleophilic substitution reaction ($S_N 2$), well-known as Williamson synthesis, proper for the synthesis of phenol ethers. In the first stage, the hydrogen hydroxyls located at the aromatic ring of bergenin are deprotonated by the basic carbonate ($K_2 CO_3$, Synth, Diadema, Brazil), generating the aroxide. Then, the nucleophilic attack on the anion’s oxygen is carried out by the specific alkyl halide (Scheme 2). Non-protic solvent (DMF) avoids the decrease of the reaction rate.

Bergenin (1)

Solid crystalline (mp 238-240 °C); [$\alpha$]$^20_D$ = -37.0° (c 0.20, MeOH), atmospheric-pressure chemical ionization (APCI/MS) (neg.) [M – H]: 327; IR (KBr) $\nu_{max}$ / cm$^{-1}$ 3423, 3389, 3247, 3204, 2951, 2896, 1701, 1613, 1464, 1348, 1234, 1092, 1071, 991, 858, 765; $^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 7.07 (s, H-9), 4.95 (d, $J$ 10.5 Hz, H-1), 4.09-3.35 (m, H-11, H-14), 3.90 (s, H-15); $^{13}$C NMR (75 MHz, CD$_3$OD) $\delta$ 165.79 (C-1), 81.34 (C-3), 74.19 (C-4), 119.37 (C-5), 152.28 (C-6), 149.38 (C-7), 142.21 (C-8), 111.04 (C-9), 117.24 (C-10), 75.58 (C-11), 71.82 (C-12), 82.98 (C-13), 62.63 (C-14), 60.93 (C-15).

8,10-Dimethylbergenin (2)

Amorphous white solid, yield of 90%; $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 3.89 (3H, s), 3.92 (3H, s), 3.93 (3H, s), 4.82 (1H, d, $J$ 10.3 Hz), 7.47 (1H, s); LR-ESIMS $m/z$, C$_{16}$H$_{20}$O$_9$ [M + Na]$^+$: 379.10, [2M + Na]$^+$: 735.21.

8-Methylbergenin (2a)

Amorphous white solid, yield 8%; $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 3.89 (3H, s), 3.90 (3H, s), 5.00 (1H, d, $J$ 10.5 Hz), 7.25 (1H, s); LR-ESIMS $m/z$, C$_{15}$H$_{18}$O$_9$ [M + Na]$^+$: 365.09, [2M + Na]$^+$: 707.14.
8,10-Didecyl-bergenin (3)
Amorphous white solid, yield 89%; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 0.95 (6H, t), 1.30-1.60 (16H, m), 1.75-1.90 (4H, m), 3.90 (3H, s), 4.82 (1H, d, $J$ 10.3 Hz), 7.45 (1H, s); LR-ESIMS $m/z$, C$_{26}$H$_{42}$O$_9$ [M + Na]$^+$: 519.32, [2M + Na]$^+$: 1015.55.

8,10-Dioctyl-bergenin (4)
Amorphous white solid, yield of 92%; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.90 (6H, t), 1.25-1.65 (24H, m), 1.75-1.85 (4H, m), 3.91 (3H, s), 4.74 (1H, d, $J$ 10.2 Hz), 7.40 (1H, s); LR-ESIMS $m/z$, C$_{20}$H$_{30}$O$_9$ [M + Na]$^+$: 575.38, [2M + Na]$^+$: 1127.70.

8,10-Dihexyl-bergenin (5)
Amorphous white solid, yield of 76%; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.90 (6H, t), 1.30-1.60 (32H, m), 1.80 (4H, m), 3.92 (3H, s), 4.82 (1H, d, $J$ 10.2 Hz), 7.45 (1H, s); LR-ESIMS $m/z$, C$_{18}$H$_{26}$O$_9$ [M + Na]$^+$: 631.46, [2M + Na]$^+$: 1239.81.

8,10-Ditetradecyl-bergenin (6)
Amorphous white solid, yield of 79%; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.88 (6H, t), 1.27-1.34 (48H, m), 1.53-1.65 (4H, m), 3.90 (3H, s), 4.73 (1H, d, $J$ 10.0 Hz), 7.38 (1H, s); LR-ESIMS $m/z$, C$_{32}$H$_{48}$O$_9$ [M + Na]$^+$: 1464.03.

Brine shrimp test
For an initial screening on the bioactivity, the adapted brine shrimp lethality (BST) assay described by Meyer et al.17 was employed to study the general cytotoxicity of the compounds 1-6. Alive brine shrimp (Artemia salina Leach) cysts (500 mg) were transferred to a conical flask containing 3500 mL of artificial seawater. The flasks were aerated with the aid of an air pump and kept at 28 °C under a bright light. The nauplii hatched after 48 h. The compounds 1-6 were separately dissolved artificial seawater and dimethylsulfoxide (DMSO, Merck, Darmstadt, Germany) to reach the final concentration of 1 mg mL$^{-1}$. Solutions of each compound (25, 50, 75, 100 and 150 μg mL$^{-1}$) were transferred to vials, which already contained seawater (5 mL) and 10 nauplii. Controls containing DMSO, seawater and 10 nauplii were also performed. The assays were carried out in triplicate. After 24 h of incubation, the number of live nauplii was counted. The mortality was defined as the absence of controlled forward motion during 30 s of observation. The lethal concentration doses for 50% of the brine shrimp (LC$_{50}$) and the respective 95% confidence intervals were determined by using the GraphPad Prism (La Jolla, CA) software.18

Evaluation of antibacterial activity
The in vitro antimicrobial activity of bergenin (1) and its derivatives (2-6) were determined by minimum inhibitory concentration (MIC) assays based on the broth microdilution method.19

*Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enterica subsp. enterica* (ATCC 6017), *Escherichia coli* (ATCC 8739), and *Staphylococcus aureus* subsp. *aureus* (ATCC 6538) were the standard strains used in the assay. Initially, the bacteria were transferred to Muller Hinton agar (Difco Labs, Detroit, USA), and individual 24-h colonies were suspended in 10.0 mL of Muller Hinton broth (Difco, Detroit, USA). A spectrophotometer (Femto, São Paulo, Brazil) at a wavelength ($\lambda$) of 625 nm was used to standardize the suspensions of each microorganism, to match the transmittance of 81, equivalent to 0.5 in the McFarland scale (1.5 × 10$^8$ colony forming units mL$^{-1}$). Dilution of the standardized suspension generated the final concentration of 5 × 10$^5$ CFU mL$^{-1}$. The samples were dissolved in DMSO at 1 mg mL$^{-1}$. Concentrations ranging from 400 to 0.195 μg mL$^{-1}$ were achieved after dilution of samples in Mueller Hinton broth (Difco, Detroit, USA). Negative controls, three inoculated wells containing DMSO at concentrations ranging from 4 to 1%, and one non-inoculated well free of antimicrobial agent were included. One inoculated well helped to test whether the broth was adequate for microorganisms to grow. The positive control was chloramphenicol (Sigma-Aldrich, St. Louis, USA) at concentrations ranging from 4 to 1%, and one non-inoculated well free of antimicrobial agent were included. One inoculated well helped to test whether the broth was adequate for microorganisms to grow. The positive control was chloramphenicol (Sigma-Aldrich, St. Louis, USA) at concentrations ranging from 400 to 0.195 μg mL$^{-1}$ were achieved after dilution of samples in Mueller Hinton broth (Difco, Detroit, USA). Negative controls, three inoculated wells containing DMSO at concentrations ranging from 4 to 1%, and one non-inoculated well free of antimicrobial agent were included. One inoculated well helped to test whether the broth was adequate for microorganisms to grow. The positive control was chloramphenicol (Sigma-Aldrich, St. Louis, USA) at concentrations ranging from 400 to 0.195 μg mL$^{-1}$ were achieved after dilution of samples in Mueller Hinton broth (Difco, Detroit, USA). Negative controls, three inoculated wells containing DMSO at concentrations ranging from 4 to 1%, and one non-inoculated well free of antimicrobial agent were included. One inoculated well helped to test whether the broth was adequate for microorganisms to grow. The positive control was chloramphenicol (Sigma-Aldrich, St. Louis, USA) at concentrations ranging from 400 to 0.195 μg mL$^{-1}$ were achieved after dilution of samples in Mueller Hinton broth (Difco, Detroit, USA). Negative controls, three inoculated wells containing DMSO at concentrations ranging from 4 to 1%, and one non-inoculated well free of antimicrobial agent were included. One inoculated well helped to test whether the broth was adequate for microorganisms to grow. The positive control was chloramphenicol (Sigma-Aldrich, St. Louis, USA) at concentrations ranging from 400 to 0.195 μg mL$^{-1}$ were achieved after dilution of samples in Mueller Hinton broth (Difco, Detroit, USA). Negative controls, three inoculated wells containing DMSO at concentrations ranging from 4 to 1%, and one non-inoculated well free of antimicrobial agent were included. One inoculated well helped to test whether the broth was adequate for microorganisms to grow. The positive control was chloramphenicol (Sigma-Aldrich, St. Louis, USA) at concentrations ranging from 400 to 0.195 μg mL$^{-1}$ were achieved after dilution of samples in Mueller Hinton broth (Difco, Detroit, USA). Negative controls, three inoculated wells containing DMSO at concentrations ranging from 4 to 1%, and one non-inoculated well free of antimicrobial agent were included. One inoculated well helped to test whether the broth was adequate for microorganisms to grow. The positive control was chloramphenicol (Sigma-Aldrich, St. Louis, USA) at concentrations ranging from 400 to 0.195 μg mL$^{-1}$ were achieved after dilution of samples in Mueller Hinton broth (Difco, Detroit, USA). Negative control...
positive LR-ESIMS molecular ions, permitted to identify all the derivatives of the bergenin. It is important to highlight that the compounds 2, 2a and 4 were previously reported, but the other derivatives (3, 5 and 6) are being described for the first time herein.

Subsequently, the bioactivities of the bergenin (1) and its derivatives (2-6) were investigated and compared to demonstrate how the alkylation of the bergenin hydroxy aromatic groups affected the bioactivity. In an initial screening, the brine shrimp lethality assay was performed and the cytotoxicities of 1-6 were evaluated based on the LC₅₀ values against *Artemia salina*. The current literature reports that a compound that displays LC₅₀ < 100 µg mL⁻¹ against *A. salina* can be considered highly toxic. While values of LC₅₀ between 100 and 1000 µg mL⁻¹ or upper than 900 µg mL⁻¹, classify the assayed compound as moderate or nontoxic, respectively. According to this, bergenin (1) did not display toxicity against *A. salina* (Table 1). On the other hand, all evaluated derivatives (2-6) displayed greater toxicity than the parent compound 1 (Table 1). It is worth highlight the toxicity displayed by the derivative 8,10-dihexylbergenin (3), which was similar to that displayed by the positive control.

Since the brine shrimp lethality assay provides a convenient pre-screening method for assessing cytotoxicity, Bergenin (1) and its semi-synthetic derivatives (2-6) could display other bioactivities. The search for compounds with antimicrobial activity has reached increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms.

The antimicrobial activity of all compounds (1-6) against a representative panel of bacteria was evaluated in terms of MIC values. The chemicals were assayed against the following bacteria: *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enterica* subsp. *enterica* (ATCC 6017), *Escherichia coli* (ATCC 8739), and
Staphylococcus aureus subsp. aureus (ATCC 6538). Chloramphenicol was the positive control and displayed MIC value of 12.5 to 0.20 µg mL\(^{-1}\) against the bacteria strains (Table 2). Our results showed that bergenin (1) was inactive against all bacteria (MIC > 400 µg mL\(^{-1}\)). However, its derivatives displayed selective activity against the Gram-positive bacteria Staphylococcus aureus. The compounds 2, 4, and 6 displayed interesting activity against S. aureus (Table 2). The antimicrobial activity of derivatives 3 and 5 against S. aureus was noteworthy once the MIC value was 3.12 µg mL\(^{-1}\) for both.

Previously, it was reported that the methanolic extract of P. dubium was able to inhibit S. aureus, either normal strain ATCC 8095 (MIC = 0.5 mg mL\(^{-1}\)) or antibiotic-resistant strain INEL 2213 (MIC = 0.25 mg mL\(^{-1}\)). In addition, the extracts of P. dubium were able to inhibit Enterococcus faecium, P. aeruginosa and Salmonella typhimurium. Our antibacterial assays pointed out for the synergism between bergenin and other chemical compounds present in the methanolic extract of P. dubium, which explains the activities reported by this previous study. In addition, the alkylation of the bergenin generated derivatives that displayed more interesting inhibitory capabilities on S. aureus than bergenin itself.

Finally, as part of our interest in expanding the knowledge about the bioactivities of the bergenin (1) and its derivatives (2-6), all compounds were submitted to the evaluation of inhibition of AChE. Phystostigmine was the positive control and displayed IC\(_{50}\) = 163.11 ± 0.39 µmol L\(^{-1}\). Among the evaluated compounds, bergenin displayed the most interesting inhibitory activity (IC\(_{50}\) = 141.19 ± 0.41 µmol L\(^{-1}\)). All the semi-synthetic derivatives of the bergenin displayed modest inhibition on AChE (IC\(_{50}\) values of 432.93 ± 0.17, 495.14 ± 0.10, 650.80 ± 0.12, 568.55 ± 0.09, and 853.98 ± 0.13 µmol L\(^{-1}\) for compounds 2-6, respectively). Our results showed that the presence of hydroxyl aromatic groups is important to inhibition of AChE by bergenin (1). In addition, the larger alkyl chains further hinder the inhibitory activity on.

Conclusions

Bergenin (1) was isolated in good yields and the semisynthetic approach used for its derivatization was successful. A total of six alkyl derivatives of the bergenin were achieved. Among them, three derivatives were reported for the first time herein, 8,10-diheptylbergenin (3), 8,10-didecylbergenin (5), and 8,10-ditetradecylbergenin (6); along with three previously reported ones, 8,10-dimethylbergenin (2), 8-methylbergenin (2a), and 8,10-dioctylbergenin (4).

Prompted by the frequent interesting biological activities displayed by the synthetic derivatives of natural products, we investigated the cytotoxicity and antibacterial activities, and inhibition on AChE of the bergenin and its derivatives. All compounds displayed low or moderate toxicity in the brine shrimp lethality assay, only 8,10-diheptylbergenin was considered toxic against this model. All compounds selectively inhibited the Gram-positive Staphylococcus aureus in the antimicrobial assay. The derivatives 3 and 5 deserve attention because they displayed very interesting inhibitory activity against S. aureus (MIC = 3.12 µg mL\(^{-1}\)). Regards the inhibition of AChE, bergenin was the most active compound and the results permitted some considerations about the importance of free hydroxy groups to such activity. Certainly, our results open new possibilities for rational guidance for the design of other bioactive derivatives of the bergenin for pharmacological studies.

Supplementary Information

Supplementary information (Figures S1-S25) is available free of charge at http://jbcs.sbq.org.br as PDF file.
Acknowledgments

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) under grant No. 406427/2018-6. The authors also thank CAPES and CNPq for the scholarships.

Author Contributions

Oscar C. Silva Neto was responsible for the data curation, formal analysis, investigation, methodology; Marla T. F. Teodoro for the investigation and methodology; Bruna O. do Nascimento for the data curation, methodology and software analysis; Klauber V. Cardoso for the data curation and methodology; Eliane de O. Silva for the data curation, software analysis; Klauber V. Cardoso for the data curation, methodology and software analysis; Oscar C. Silva Neto was responsible for the data curation, formal analysis, investigation, methodology; Eliane de O. Silva for the data curation, methodology and software analysis; Jorge M. David conceptualization, data curation, formal analysis, supervision, writing draft and editing; Juceni P. David for the project administration, resources, supervision, writing analysis, funding acquisition, investigation, methodology, and methodology; Marla T. F. Ferreira for the investigation and methodology; Teodoro for the investigation and methodology; Bruna O. do Nascimento for the data curation, methodology and software analysis; Klauber V. Cardoso for the data curation, formal analysis, supervision, writing draft and editing; Juceni P. David for the conceptualization, writing original draft, review, funding acquisition.

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Submitted: March 11, 2020
Published online: July 9, 2020

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