Identification of Three New Phenolic Compounds from *Ozoroa obovata* (Anacardiaceae) Root

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ABSTRACT

In recent years, interest in medicinal plants has grown considerably, particularly in African species, which play a key role in primary healthcare and represent a largely underexplored source of bioactive compounds. *Ozoroa obovata* (Anacardiaceae) is traditionally used in southern Africa to treat various conditions, including stomachache, sexually transmitted diseases, fever, diarrhea, and respiratory infections. However, its chemical composition and biological activities remain poorly investigated. This study aimed to investigate the phytochemical composition of the ethyl acetate partition obtained from the roots of *O. obovata*, guided by biological assays. Three phenolic compounds, methyl gallate, fustin, and taxifolin, were isolated and identified using ESI-HRMS and 1D and 2D NMR analyses, supported by comparison with literature data. Previous evaluations of ethanolic extracts from twigs, leaves, and roots demonstrated antimicrobial activity with the ethyl acetate partition showing notable effects. The isolation of methyl gallate (I), fustin (II), and taxifolin (III) is noteworthy, as these compounds have been reported in the literature to exhibit significant pharmacological activities in different plant extracts. This is the first report of these compounds in the roots of *O. obovata* ethyl acetate partition fraction. These findings expand the phytochemical knowledge of the species and highlight its potential as a source of bioactive compounds with pharmacological relevance.

KEYWORDS

fustin, isolated compounds, methyl gallate, *Ozoroa obovata*, phenols, phytochemicals, root, taxifolin

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INTRODUCTION

Ozoroa obovata is a species of the genus *Ozoroa* (Anacardiaceae), primarily distributed in southern Africa. The genus is recognized for its wide diversity of bioactive constituents and its extensive use in traditional medicine. Phytochemical investigations have revealed the presence of structurally diverse specialized metabolites (also known as secondary metabolites), which are biosynthetically derived compounds typically involved in plant defense and ecological interactions, although their specific functions are not always fully elucidated. These metabolites include phenolic acids and their derivatives, many of which exhibit relevant biological activities such as antimicrobial, anti-inflammatory, antioxidant, antimalarial, and antitumor effects. Such properties highlight the pharmacological potential of the genus and support its ethnopharmacological applications.[1]

In recent years, there has been a growing interest in medicinal plants, particularly those from African flora, which play a crucial role in primary healthcare systems and represent a valuable yet underexplored source of bioactive compounds. In this context, emphasis has been placed not only on the traditional uses of the African plants but also on their chemical composition and biological potential,[2] underscoring, within this botanical diversity, the relevance of the genus *Ozoroa*.[1]

Comprehensive phytochemical studies of extracts obtained from the and leaves of *O. obovata* have revealed multiple classes of specialized metabolites. Analyses by HPLC-ESI-MS noted phenolic acids, long-chain phenolic acids, particularly anacardic acids, and their derivatives, as well as flavonoid heteroside and aglycones.[3,4] These findings demonstrate the chemical complexity of this species and reinforce its potential as a source of bioactive natural products. Furthermore, the metabolites of *O. obovata* have already been associated with biologically relevant activities, making them important targets for drug discovery.[5,6]

Considering previous antimicrobial activity studies that guided the investigation of root-derived metabolites, the phytochemical profiles of different parts of *O. obovata* likely contribute to the antibacterial and antifungal effects reported for this species. The ethanolic extracts of *O. obovata* leaves and twigs demonstrated moderate antibacterial activity against both Gram-positive and Gram-negative oral microorganisms, as well as activity against *Candida albicans, Candida glabrata,* and *Candida tropicalis,*[3,4] suggesting that, even in their unrefined form, these extracts contain compounds capable of exerting antimicrobial effects. When the ethanolic extract and root-derived partitions of *O. obovata* were evaluated against these microorganisms, promising antifungal potential was observed against the yeasts, with the ethyl acetate partition showing the most pronounced activity.[7] Moreover, the selectivity index (SI) values indicated that the extract and its partitions exhibit preferential toxicity toward fungal cells compared to the tested Vero cells. These evaluations revealed the notable anti-*Candida* activity of the ethyl acetate partition, supporting its selection for further phytochemical investigation.

Most phytochemical studies on *O. obovata* have focused on aerial parts, with limited attention given to root-derived fractions. In particular, the chemical composition of the ethyl acetate partition obtained from the roots remains poorly explored, and information regarding its bioactive constituents is still scarce. This gap highlights the need for further studies aimed at the identification and characterization of compounds from this partition. Accordingly, the present study reports a bioassay-guided phytochemical investigation of the ethyl acetate root partition of *O. obovata*. This approach led to the isolation and structural characterization of three compounds, methyl gallate (**I**), fustin (**II**), and taxifolin (**III**).

The identification of these phenolic constituents contributes to the phytochemical characterization of the species and provides

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a foundation for future investigations into structure–activity relationships and potential synergistic effects within the extract. To the best of our knowledge, this study represents the first report on the isolation of chemical constituents from the ethyl acetate root partition of *O. obovata*, underscoring its originality and scientific relevance.

EXPERIMENTAL

Standard chemicals

All reagents, solvents, and standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant material

The species was identified and authenticated by Dr. Eugénio Augusto Chilengue, and a voucher specimen (No. 18) was deposited in the Herbarium of the Institute of Traditional and Alternative Medicine, Misau, Maputo, Mozambique. The roots were air-dried under ambient conditions for ten days and subsequently transported to Brazil for analysis at the Institute of Chemistry, Federal University of Uberlândia (UFU).

Extraction and purification of compounds

Dried roots of *O. obovata* (Oliv.) R. Fern. & A. Fern. (Anacardiaceae) (295 g) were subjected to maceration in 99% ethanol, for 48 h, at room temperature. The resulting mixture was then filtered to obtain the crude methanolic extract. The extraction process was repeated five times, and the combined extract was concentrated under reduced pressure and lyophilized (76.0 g). The dried residue was then subjected to liquid–liquid partitioning using solvents of increasing polarity.

Liquid-liquid partitioning and fractionation of EAP

Approximately 15.0 g of ethanolic root extract (EER) were resolubilized in 100.0 mL of methanol-water (9:1, v/v) and partitioned using the following sequence of solvents: n-hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and *n*-butanol (5 × 200.0 mL each). This procedure resulted in the following partitions: n-hexane (HP, 0.8 g; 5.3%), dichloromethane (DP, 1.0 g; 6.6%), ethyl acetate (EAP, 3.0 g; 20.0%), n-butanol (BP, 5.0 g; 33.3%), and the remaining aqueous partition (WP, 12.0 g; 85.3%). Each partition was concentrated in a rotary evaporator and lyophilized to ensure complete solvent removal before further analysis (Figure 1).[8]

EAP (1.0 g) was subjected to silica gel column chromatography (63–200 µm, 70–230 mesh; 36.5 g, 21.5 × 2.5cm i. d. The elution of the column utilized a gradient starting from *n*-hexane-ethyl acetate (9:1, v/v) progressing to pure ethyl acetate, which was then followed by a transition to ethyl acetate-methanol (1:9, v/v) leading to pure methanol. A total of 104 fractions were collected, evaluated by thin layer chromatography (TLC), and observed under ultraviolet light (UV 254 or 365 nm). The fractions obtained during chromatographic separation were carefully monitored by TLC under UV light (254 and 365 nm) and after derivatization with NP/PEG reagent (Naturstoff reagent/polyethylene glycol) and sulfuric vanillin. Based on their TLC profiles, the eluates were combined to yield 17 pooled fractions. Among those collected fractions, EAFr45 (37.8 mg) was noted to be predominantly pure, highlighting its significance for further isolation procedures. Consequently, it was subjected to HPLC (high performance liquid chromatography) analysis to detail the chemical characteristics of EAFr45.

Fractionation of EAFr45

EAFr45 (37.8 mg) was fractionated using a Shimadzu HPLC system (LC–6AD) equipped with a degasser (DGU–20A5R), column oven (CTO–20A), autosampler (SIL–10AF), fraction collector (FRC–10A), diode array detector (DAD, SPD–M20A), and evaporative light scattering detector (ELSD, LT II), all controlled by LC Solution software.

Analytical separation was performed on a C18 reversed-phase column (250 mm × 4.6 mm, 5 µm; Phenomenex Luna) with a corresponding guard column. The sample (1 mg mL^{-1} in methanol) was analyzed using a mobile phase consisting of deionized water (A) and HPLC-grade methanol (B). The gradient elution was as follows: 5% B (0 min), 35% B (5 min), held at 35% B (7 min), increased to 50% B (15 min), held until 17 min, increased to 100% B (22 min), held until 25 min, and then returned to 5% B (30 min). The flow rate was maintained at 1 mL min^{-1}, with an injection volume of 50.0 µL of the sample solution. UV detection was performed at 366 nm, and the column oven temperature was maintained at 35 °C.

Based on the analytical profile, EAFr45 (37.8 mg; 20 mg mL^{-1} in methanol) was further purified by semi-preparative HPLC using a C18 column (250 × 10 mm, 10 µm; Phenomenex Luna). The mobile phase consisted of water (A) and HPLC-grade methanol (B), using the same gradient elution as in the analytical separation. The flow rate was 2.0 mL min^{-1} with an injection volume of 200.0 µL. Fractions were automatically collected based on retention times (t_R), yielding **I** (3.8 mg, t_R = 14.51 min), **II** (4.4 mg, t_R = 17.71 min), and **III** (4.5 mg, t_R = 18.95 min) as amorphous solid compounds (Figure S1).

Mass spectrometry analysis (HPLC-ESI-MS)

Compounds **I**–**III** were analyzed by HPLC–HRMS using an Agilent 1260 Infinity system coupled to a quadrupole time-of-flight (Q-TOF) mass spectrometer (Agilent 6520) equipped with an electrospray ionization (ESI) source. Separation was performed on a reversed-phase C18 column (100 × 2.1 mm, 2.7 µm; Agilent Zorbax). The injection volume was 2.0 µL. The mobile phase consisted of 0.1% (v/v) formic acid in Milli-Q water (A) and methanol (B). Elution was carried out at a flow rate of 0.08 mL min^{-1} using the following gradient program: 10% B (0 min), increased to 98% B over 10 min, and held at 98% B until 17 min. The ionization parameters were as follows: nebulizer gas pressure, 58 psi; drying gas flow, 8 L min^{-1} at 220 °C; and capillary voltage, –4.5 kV. High-purity nitrogen (99.9%) was used as both drying and collision gas. Data were acquired in negative ion mode over the mass range *m/z* 50–1000. Molecular ions were further subjected to MS2 experiments at different collision energies.

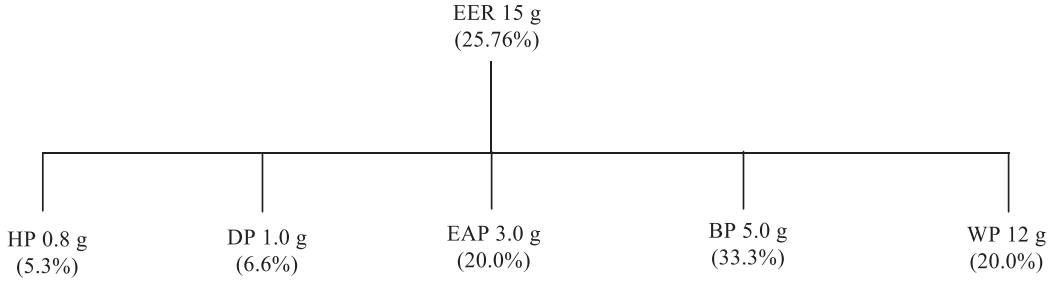


Figure 1: Extract yield and liquid-liquid extraction. Note: EER= root ethanolic extract, HP= *n*-hexane partition, DP= dichloromethane partition, EAP= ethyl acetate partition, BP = *n*-butanol partition, WP= water partition.

The molecular formulas of compounds **I**–**III** (methyl gallate, fustin, and taxifolin, respectively) were established based on accurate mass measurements and fragmentation patterns, in comparison with literature data and the spectra is provided in the Supplementary Material.

NMR analysis

Compounds **I**–**III** were analyzed by NMR spectroscopy using a Bruker Ascend™ 400 Avance III HD spectrometer (9.4 T), operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. All experiments were performed at 30°C. The samples were dissolved in CD_3OD (deuterated methanol), using tetramethylsilane (TMS, δ = 0.00 ppm) as internal standard. All NMR data is available in the Supplementary Material. The following spectra were acquired: ^{1}H NMR, ^{13}C NMR, and DEPT, as well as two-dimensional experiments including COSY, HSQC, and HMBC. The corresponding spectra are presented in the Supplementary Material (Figures S1–S20).

RESULTS AND DISCUSSION

Based on bioassay-guided evaluation of *O. obovata* root extracts, the ethyl acetate fraction, was selected for further phytochemical investigation to identify the compounds responsible for the observed effects.

Chemical characterization of compounds isolated from the roots of *O. obovate*

Methyl gallate (**I**), fustin (**II**), and taxifolin (**III**) are polyphenolic compounds isolated from the roots of *O. obovata* (Anacardiaceae) and are reported for the first time in this genus. Their chemical structures (Figure 2) were established by HPLC, ESI–MS, and NMR analysis.

Compound I

Methyl gallate (**I**), a phenolic derivative of gallic acid, exhibits diverse biological activities including antioxidant, anti-inflammatory, and anticancer effects.[8] Its antitumor activity is mainly associated with induction of apoptosis through caspase activation, modulation of Bcl-2 family proteins, ROS generation, and inhibition of tumor angiogenesis.[9]

The compound **I** appeared as a yellowish-white, amorphous solid powder, exhibiting UV-vis absorption with a λ_{max} band at 254 nm, and a retention time (t_R) of 14.51 min in the HPLC chromatogram (Figure S1). A deprotonated molecular ion $[M - H]^-$ at *m/z* 183.0309 (Δ = −5.4 ppm) was observed in the HR–ESI–MS (negative mode; Figure S2), along with the analysis of the ^{1}H and ^{13}C NMR spectra, suggesting the molecular formula $C_8H_8O_5$, indicating five degrees of unsaturation.

The ^{1}H NMR spectrum of compound **I** exhibited a simple signal pattern characteristic of a phenolic compound, including aromatics with signals of a singlet at δ 6.94 (H-2 and H-6; 2H) symmetric to themselves and a singlet at δ 3.72 (-CH_3; 3H) (Figure S3; Table S1).

The ^{13}C NMR spectra exhibited six spectral signals in the range corresponding to the aromatic ring: a signal at δ 110.29 (C-2 and C-6; 2C), δ 121.65 (C-1; 1C), a para signal at δ 140.07 (C-4; 1C), and a meta signal at δ 146.73 (C-3 and C-5; 2C). The signal at δ 169.28 (C=O) and at δ 52.47 (-OCH_3) of a methoxy group (Figure S4, Table S2). DEPT showed two signals: a CH at δ 110.29 corresponding to the asymmetric carbons (C-2 and C-6; 2C) and another at δ 52.47 (-OCH_3) to the methoxy group (Figure S5, Table S2). A ^{13}C resonance at δ 171 corresponds possibly to dimethylformamide (DMF), a common impurity resulting from the extraction process. It exhibits a carbonyl signal at δ 171–173 and a formyl proton singlet at δ 8.0–8.5, with HSQC spectra (Figure S6) correlation confirming the connectivity, consistent with the well-known NMR characteristics of DMF. Thus, the combined evidence from ^{1}H and ^{13}C NMR spectra, mass spectrometry, and comparison with literature data confirmed that compound **I** is methyl gallate, a phenolic compound.[10,11] Although methyl gallate is a widely known and a documented compound in the literature this is the first record of its isolation from the roots of *O. obovata* (Anacardiaceae), as well as the first report for the genus *Ozoroa*.

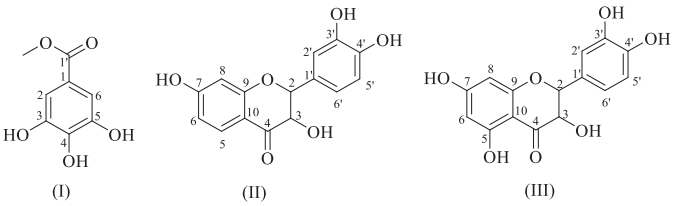


Figure 2: Chemical structures of methyl gallate (**I**), fustin (**II**), and taxifolin (**III**).

Compound II

Fustin (**II**), a flavanonol widely distributed in species such as *Rhus verniciflua* (Anacardiaceae), exhibits diverse pharmacological properties including antioxidant, anti-inflammatory, anticancer, and antidiabetic activities, mainly attributed to its ability to scavenge reactive oxygen species and modulate inflammatory and apoptotic signaling pathways.[12,13,14]

The compound **II** was obtained as a yellowish amorphous solid, showing UV–vis absorption bands at 263 and 350 nm, and a retention time (t_R) of 17.71 min in the HPLC chromatogram (Figure S1). High-resolution ESI-HRMS analysis (negative ion mode) revealed a deprotonated molecular ion $[M - H]^-$ at *m/z* 287.0526 (Δ = −1.1 ppm, $C_{15}H_{11}O_6$) (Figure S7).

The ^{1}H NMR spectrum displayed six aromatic proton signals: δ 6.89 (1H, d, *J* = 2.1 Hz, meta), 6.76 (1H, dd, *J* = 8.1, 2.1 Hz, ortho/meta), 6.71 (1H, d, *J* = 8.1 Hz, ortho), 6.42 (1H, dd, *J* = 8.7, 2.2 Hz), and 6.22 (1H, d, *J* = 2.3 Hz, meta). Two additional downfield signals at δ 4.83 (1H, d, *J* = 11.7 Hz, H-2) and 4.37 (1H, d, *J* = 11.7 Hz, H-3) correspond to protons attached to oxygenated sp^3 carbons. Thus, the chemical shift pattern observed for compound **II** shows similarity with a tetrasubstituted flavonoid nucleus at positions 3, 7, 3', and 4' (Figure S8, Table S3).

Considering the ^{13}H NMR data, it is evident that compound **II** is a flavonoid. The pattern observed in the ^{13}C NMR spectrum showed fifteen signals, including a carbonyl carbon at δ 194.40 (C-4), twelve aromatic carbons between δ 167.60 and 103.82, and two oxygenated sp^3 carbons at δ 85.61 (C-2) and δ 74.56 (C-3) (Figure S9, Table S4). The combined evidence from ^{1}H and ^{13}C NMR spectra, mass spectrometry, and comparison with literature data[15] confirmed that compound **II** is the flavonoid fustin.

To corroborate the proposed structure of compound **II**, its DEPT-135 NMR spectrum was also analyzed. This spectrum revealed eight signals in the range from δ 130.15 to 74.57, corresponding to carbons with C-H bonds (Figure S10). Among them, the signals at δ 85.61 and δ 74.57 (C-2 and C-3) were also identified in the ^{13}C NMR spectrum. When comparing the ^{13}C NMR spectrum with DEPT-135, the absence of signals in the displacement range between δ 167.60 and 146.60 is noted, as well as the signal at δ 194.40 (C-4), which is attributed to a carbonyl carbon. These data reinforce the proposed identification of compound **II** (Tables S3 and S4). The NMR spectra analysis of fustin showed signals that establish a trans relationship between H-2 and H-3. However, it is not possible to determine the absolute configuration based solely on these NMR data.

Although fustin is a flavonoid widely described in the literature, its record in the genus *Ozoroa* (Anacardiaceae) has not yet been reported. This study describes, for the first time, its isolation from the roots of *O. obovata*. Previous studies highlight the biological potential of fustin, including its ability to inhibit free radicals and anti-apoptotic properties.[16] In addition, it was reported that fustin has a gastroprotective effect[17] from its isolation from the heartwood of *Cotinus coggygria*, at concentrations between 0.10 and 0.45 μg L^{-1}. It has demonstrated a protective effect against arthritis and oxidative stress and neuroprotective effects.[18,19] Another recent study demonstrated that fustin significantly reduced the proliferation of cancerous cells in

breast and colon cancer cell lines, with inhibitory concentration values (IC_{50} = 56.02 μg L^{-1} and 78.07 μg L^{-1}, respectively).[20]

Compound III

Taxifolin (dihydroquercetin) is a naturally occurring flavanonol that has attracted considerable attention due to its broad spectrum of biological activities. It has been reported to possess potent antioxidant properties, acting as an efficient scavenger of reactive oxygen species and inhibitor of lipid peroxidation. In addition, taxifolin exhibits anti-inflammatory, anticancer, cardioprotective, hepatoprotective, and neuroprotective effects, which have been associated with the modulation of signaling pathways related to oxidative stress, inflammation, and apoptosis.[21,22]

The compound **III** appeared as a yellowish amorphous solid with UV-vis absorption bands at 263 and 350 nm, and a retention time (t_R) of 18.95 min in the HPLC chromatogram (Figure S1). High-resolution ESI-HRMS analysis (negative ion mode) of compound **III** revealed a deprotonated molecular ion $[M - H]^-$ at *m/z* 303.0494 (Δ = −2.7 ppm; $C_{15}H_{11}O_7$) (Figure S14).

The ^{1}H NMR spectrum exhibited five aromatic proton signals: a doublet at δ 6.96 (H-2'; *J* = 2.1 Hz) in meta, a doublet at ortho and meta, respectively, a doublet at δ 6.84 (H-6'; *J* = 8.1-2.0 Hz; 1H), a doublet at δ 6.80 (H-5'; *J* = 8.1 Hz; 1H) in ortho position, a doublet at δ 5.90 (H-6; *J* = 2.1 Hz; 1H), and a doublet at δ 5.87 (H-8; *J* = 2.1 Hz; 1H) in meta positions on the aromatic ring (Figure S15, Table S5). Furthermore, two spectral signals, a doublet at δ 4.90 (H-2; *J* = 11.4 Hz; 1H) and a doublet at δ 4.39 (H-3; *J* = 11.4 Hz; 1H), were observed and are attributed to hydrogens bonded to oxygen-containing sp^3 carbons. Overall, the chemical shift pattern is consistent with a 3,7,5,3',4'-tetrasubstituted flavonoid nucleus, analogous to compound II.

The DEPT-135 NMR spectrum of compound **III** displayed seven C–H-bearing carbon signals, including resonances at δ 73.79 and 85.21 ppm, which were also observed in the ^{13}C NMR spectrum (Figures S16 and S17; Tables S5 and S6). Analysis of the ^{13}C and DEPT-135 NMR spectra indicated that the signal at δ 198.25 ppm (C-4), consistent with a carbonyl carbon, is absent in the DEPT-135 spectrum, corroborating the identification of compound **III** as taxifolin.[23,24] The ^{13}C and ^{1}H NMR spectra of taxifolin indicated signals that establish a trans relationship between H-2 and H-3, like fustin. However, it is not possible to determine the absolute configuration based solely on these NMR data.

Literature reviews emphasize the pharmacological potential of taxifolin, including antioxidant, anti-inflammatory, hepatoprotective, anti-Alzheimer's, antiangiogenic, and anti-hyperglycemic activities, its involvement in glucose homeostasis, diabetic retinopathy, and cataract prevention.[25,26] Accordingly, taxifolin is considered an important dietary bioactive compound with multiple health benefits, highlighting the significance of its isolation in the present study.

CONCLUSION

Bioassay-guided fractionation of the ethyl acetate root fraction of *O. obovata* led to the isolation of three phenolic compounds, methyl gallate, fustin, and taxifolin, whose structures were elucidated by HPLC–MS and NMR spectroscopy. These constituents were isolated from this plant for the first time. Overall, this study expands the phytochemical knowledge of *O. obovata* species and provides a foundation for further investigations into their pharmacological potential and possible synergistic effects.

SUPPLEMENTARY INFORMATION

Supplementary material is available in a separate document.

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DECLARATION OF COMPETING OR FINANCIAL INTERESTS

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

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES

The authors declare that, during the preparation of this manuscript, AI-assisted tools were used solely for language and grammar revision. All outputs were critically reviewed and edited by the authors, who assume full responsibility for the content.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Frank dos S. Silva: Writing – Original draft, Investigation, Data curation, Conceptualization. Bárbara F. de Jesus: Investigation, Data curation. Domingos A. João: Investigation, Data curation. Luis Carlos S. Cunha: Investigation, Supervision, Formal analysis. Alberto de Oliveira: Resources for Investigation, Revision, Data curation. Sergio A. L. Morais: Supervision, Formal analysis, Conceptualization, Data curation. Francisco J. T. Aquino: Conceptualization, Supervision, Review & editing, Investigation, Formal analysis.

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