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Perimidines Synthesis: A Simple and Effective Experiment of Organic Chemistry for Undergraduate Students

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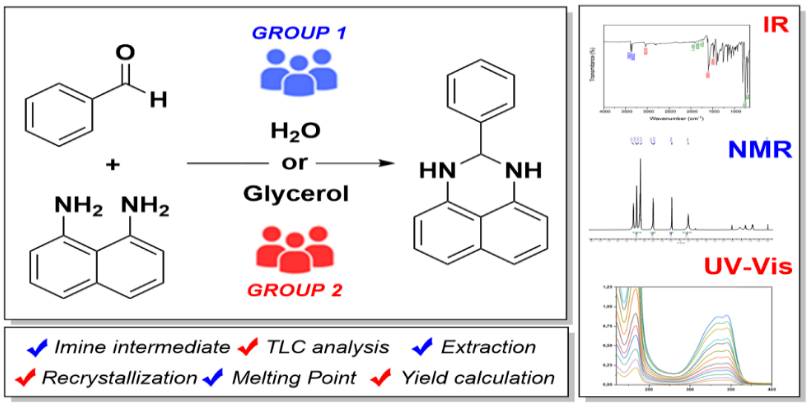
Practical laboratory experiments are essential to connecting theory to practical application and providing important support for academic training; however, many curricula still lack effective practical activities. This work proposes a didactic laboratory experiment highlighting the organic synthesis of a heterocyclic perimidine, providing an integrative approach to concepts such as carbonyl addition followed by elimination of water (imine formation), thin-layer chromatography (TLC), liquid-liquid extraction, recrystallization, and yield calculations. Furthermore, a comparison was made between water and glycerol as environmentally friendly (green) solvents. Characterization techniques such as melting point, spectroscopy NMR ^{1}H and ^{13}C, vibrational spectroscopy (FTIR) and UV-Vis were also used for product identification and discussion. The experiment was designed for undergraduate students and offered an opportunity to explore a variety of concepts in organic chemistry while integrating sustainable practices with fundamental techniques, demonstrating its potential as an effective and engaging educational tool.

Graphical abstract



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1. Introduction

Didactic laboratory experiments play a crucial role in bridging the gap between theoretical knowledge and practical skills [1-3]. By offering hands-on experience, they enhance students' understanding of fundamental concepts in organic chemistry [4,5]. Despite the central role of laboratory practice in chemistry education, many didactic experiments rely on outdated or overly simplified synthetic pathways that fail to reflect the diversity and complexity of real-world organic

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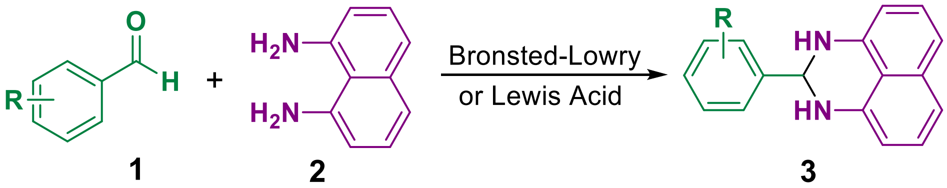
synthesis [6,7]. Furthermore, they often neglect the incorporation of contemporary strategies and sustainability principles [8].

This lack of variety not only limits student engagement but also overlooks opportunities to introduce innovative and relevant practices [9]. There is, therefore, a need for accessible experiments that combine fundamental organic reactions with the synthesis of biologically relevant molecules, for example. Among the diverse classes of compounds that can be explored in this context are heterocycles, such as perimidines.

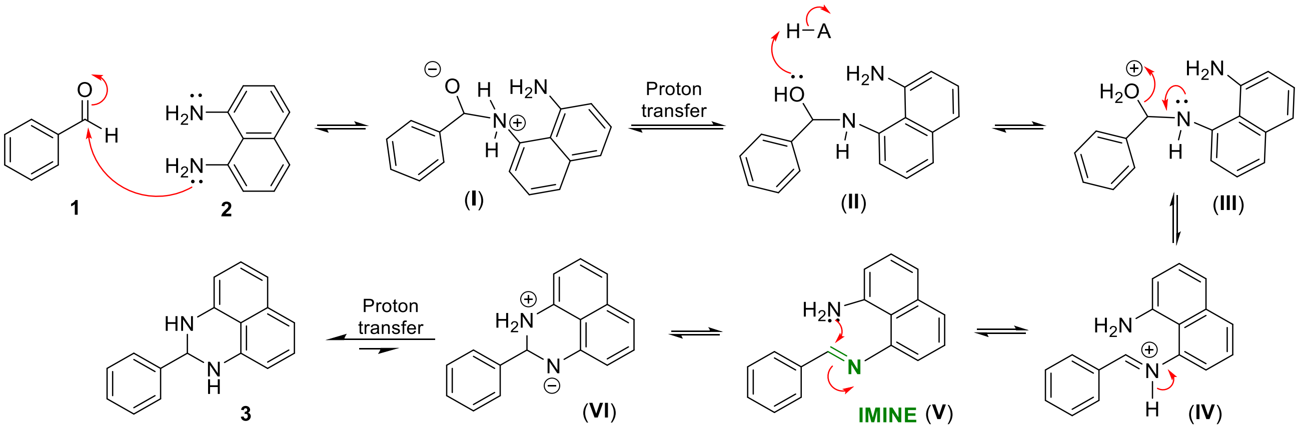
Perimidines are a group of heterocyclic molecules that are being extensively explored due to their capabilities across various applications, for example, as chemical sensors [10,11], dyes [12], organic hydrates [13], and its biological activity, in particular, anticarcinogen [14,15], antimicrobial [16], anti-inflammatory [17] and antioxidant [18]. Also, in the field of drug discovery and enhancement it is well known that heterocyclic moieties are commonly found in the structure of molecules that exhibit biological activity [19,20], elucidating the value of synthesizing this class of molecules.

In the literature, the most reported route to obtain 2-substituted 2,3-dihydro-1*H*-perimidines **3** is through the condensation reaction of aldehydes **1** with 1,8-diaminonaphthalene **2**, commonly utilizing a Bronsted-Lowry or Lewis acid as a catalyst (Scheme 1) [21,22].



Scheme 1. Perimidines condensation reaction.

As illustrated in Scheme 2, the initial steps in the mechanism of perimidine synthesis catalyzed by acid involve the intermolecular nucleophilic addition of amine from 1,8-diaminonaphthalene **2** to benzaldehyde **1** to generate intermediate **I**, followed by a proton transfer to form a neutral carbinolamine intermediate **II**, which is protonated by acid (intermediate **III**) to eliminate water (dehydratation) and obtain the iminium ion **IV**. The iminium ion **IV** is deprotonated to produce the imine **V**, also known as Schiff base, highlighted in green. The following step is an intramolecular nucleophilic attack of the other amine to imine **V**, leading to the cyclization of the heterocyclic ring and, finally, a proton transfer of the intermediate **VI**, resulting in the perimidine **3**. This mechanistic pathway can be seen in Scheme 2 and provides a comprehensive discussion of imine formation, as well as its properties and role within the reaction mechanism.



Scheme 2. Perimidines acid-catalyzed reaction mechanism via nucleophilic addition to the carbonyl, followed by water elimination (imine formation).

A valuable pedagogical aspect of this reaction lies in the synthesis and reactivity of imines, a fundamental functional group typically introduced to undergraduate students in Organic Chemistry, particularly during their sophomore year. They serve as an example of nucleophilic addition at the carbonyl group with the elimination of water, as presented in the Clayden textbook [23]. Hence, applying imine (**V**) as an organic scaffold is important to comprehend the pathway to synthesize more complex molecules, for example, heterocyclic compounds like perimidines. Although synthesis experiments of imines for undergraduate students already exist in the literature [23-25], no studies focus on the synthesis of perimidines using this intermediate, which expands essential concepts and improves pedagogical scope.

Given the technical and didactic importance of perimidine synthesis, we propose an experimental session in organic synthesis designed for undergraduate students in chemistry, pharmacy and engineering courses. This work describes a straightforward experiment to synthesize and characterize the heterocyclic compound 2-phenyl-2,3-dihydro-1*H*-perimidine **3** in a laboratory setting, using benzaldehyde **1** and 1,8-diaminonaphthalene **2**, inexpensive and readily available reagents. Among the various synthetic routes reported for the synthesis of perimidines, this one stands out for its simplicity, efficiency, use of non-toxic solvents, and mild reaction conditions, such as for reactions carried out in a water or glycerol medium [26, 27].

Notably, the direct use of water or glycerol as reaction media demonstrates a sustainable approach suitable even for undergraduate laboratory experiments, as these are considered green solvents; moreover, glycerol is an abundant byproduct of biodiesel production, which has led to increased interest in its valorization as a green solvent in organic synthesis [28].

The synthesis and characterization proposed in this work are structured into two sessions, each lasting three hours. The reactions involved provide valuable opportunities for students to explore fundamental concepts in the chemistry of carbonyl compounds, such as carbonyl addition and the reactivity of imines. Additionally, this experiment introduces students to important laboratory techniques employed in organic synthesis, including TLC reaction monitoring, liquid-liquid extraction, rotary evaporation, recrystallization and melting point determination, in addition to spectroscopic characterization techniques essential for the structural elucidation of organic compounds, such as IR, NMR, and UV-Vis.

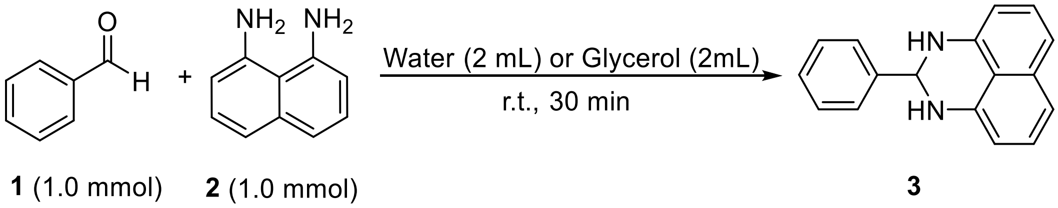
2. Material and Methods

2.1 General Information

All starting materials, reagents and solvents were commercially available and used as received. Thin-layer chromatography (TLC) was performed using Macherey-Nagel GF254, 0.20 mm thickness. For the visualization, TLC plates were either placed under UV light (254nm) and stained with iodine vapour. ^{1}H and ^{13}C NMR spectra were taken on a Bruker DPX-300 Avance spectrometer, with $CDCl_3$. As noted ^{1}H NMR spectra were referenced to 0.00 ppm (TMS), or 7.26 ppm ($CDCl_3$), and ^{13}C NMR spectra were referenced to 77.16 ppm ($CDCl_3$). All chemical shifts are reported in parts per million (ppm) and coupling constants (*J*) are expressed in Hertz (Hz). Fourier-Transform Infrared Spectroscopy (FT-IR) data were acquired with infrared from Perkin Elmer, Frontier model with Attenuated Total Reflectance (ATR), from 4000 to 650 cm^{-1}, in the mid-infrared region. UV-Vis analysis were performed in a Shimadzu UV-1900 spectrometer, using quartz cuvettes with an optical path of 1 cm, in the region 190 to 1100 nm. A stock solution of 1.0 x 10^{-3} mol.L^{-1} in ethanol was prepared to perform the dilutions of the different concentrations of the product. Information about Chemical Hazards are available in the Support Information.

2.2 Lab Session 1: synthesis, extraction and recrystallization of the product

The experiment was applied to a third-year chemistry undergraduate class (sophomore), in the experimental organic chemistry discipline. Initially, the students received a pre-questionnaire about the concepts involved in the experimental class and, later, attended a brief theoretical introduction class to the experiment and the concepts yet to be explored. Along with this, they read the scripts together with the teacher to clarify any questions. After reading, the class was divided into four groups: two were assigned to use glycerol as the reaction solvent, while the other two conducted the experiment using water. The students carried out the stoichiometric calculations necessary to perform the synthesis of perimidine **3** (Scheme 3). The details of the experimental procedure for step 1 are described in the Supporting Information.



Scheme 3. Perimidine condensation reaction.

The progress of the reaction was monitored by TLC until the starting materials (benzaldehyde **1** and 1,8-diaminonaphthalene **2**) had been consumed the product formed. After the reaction was complete, the product was extracted with ethyl acetate, and the solvent was removed by vacuum. Finally, the product was recrystallized in ethanol and water.

2.3 Lab Session 2: characterizations

In the second session, the students removed the solid formed from the filter paper and calculated the yield of product **3**. To determine the purity product, a TLC analysis was performed using the product and the reagents for comparison, and the retention factor (Rf) of the product was calculated (Figure S1). Further, the melting point (m.p.) was determined using the capillary method (details in the Support Information). Spectroscopic techniques of ^{1}H NMR and ^{13}C NMR, FT-IR, and UV-Vis were used to characterize the product obtained. The spectra of the 2-phenyl-2,3-diaminenaphtalene-1*H*-perimidine **3** and the analysis and discussion of their signals are presented in the Support Information. The molar absorptivity coefficient (ε) was determined by UV-Vis analyses of product **3** in different concentrations (0.00459 at 0.0642 mmol.L^{-1}) and calculated using the Beer-Lambert Law (Equation S1).

At the end of the class, students answered the pre-questionnaire given at the beginning of the first class again, as well as a satisfaction survey about the two experimental classes. In addition, they also received a final questionnaire about the discussion of the experiment itself (Support Information).

3. Results and Discussion

3.1 Pedagogical Goal

The experiments were designed to introduce the students, specifically sophomores in chemistry and pharmacy, to the concepts of organic synthesis, emphasizing the relationship between forming carbon-nitrogen bonds in the laboratory and their application in the industry. Moreover, since it is a simple experiment, it helps develop the skills required to perform practical organic chemistry procedures. The experiment is designed to achieve the following pedagogical goals:

- Understand and apply key organic reactions, such as carbonyl addition, dehydration and imine formation, which are foundational in organic synthesis.
- Discuss the industrial and biomedical significance of heterocyclic compounds, connecting the experiment to real-world applications in pharmaceutical and materials science.
- Consider the implications of utilizing different solvents in chemical reactions, including the use of greener solvents such as water and glycerol.
- Gain experience in reaction monitoring through techniques such as thin-layer chromatography (TLC), ensuring students understand the concept of reaction progress and product verification.
- Learn the essential purification techniques, including liquid-liquid extraction, recrystallization, and the use of a rotary evaporator, emphasizing their importance in both academic and industrial settings.
- Practice calculation of reaction yields.
- Learn to characterize the product by melting point (m.p.) and spectroscopic techniques such as ^{1}H NMR, ^{13}C NMR, FTIR and UV-Vis (molar absorptivity) and discuss the results.

3.2 Experimental Overview

Participation in this experiment required students to have completed the theoretical organic chemistry course, making this activity suitable for those enrolled in Experimental Organic Chemistry. Before the experiment, students were provided with detailed experimental protocols, which included both the practical procedures and a brief theoretical explanation of the processes involved. These materials were made available in advance to allow students to familiarize themselves with the content outside of class, ensuring the majority of class time could be dedicated to hands-on activities. The protocols are included in the Supporting Information.

Additionally, a pre-experiment questionnaire was distributed at the start of the first session to assess students' prior knowledge about the main concepts covered in the first experimental session, such as stoichiometric calculations, carbonyl reactivity, nucleophilic addition and water elimination reaction mechanisms of the carbonyl group, solubility, chromatography, filtration, and recrystallization.

A total of eight students participated in the experiment, divided into four groups. Two groups were assigned to use water as the reaction medium, while the other two used glycerol to synthesize the product **3**. Students began by performing the necessary stoichiometric calculations, followed by the weighing of reagents. The synthesis began with the solubilization of 1,8-diaminonaphthalene **2** (nucleophile) in the respective solvent (glycerol or water), at room temperature, followed by the addition of benzaldehyde **1** (electrophile). In this step, the students were able to observe that the nucleophilic diamine **2** is more soluble in glycerol than in water. After the addition of the electrophile, the reaction progress was monitored by thin-layer chromatography (TLC) until complete consumption of the starting materials (**1** and **2**) and formation of the desired product **3**.

Upon confirming the reaction's completion, students carried out liquid-liquid extraction using ethyl acetate and water. Next, the organic layers were dried with anhydrous sodium sulfate, filtrated into a round-bottom flask, and concentrated using a rotary evaporator. Once dried, the crude product was solubilized in ethanol and the solution was placed into an ice-water bath to recrystallize the desired product. Then, the crystals were filtered through a simple funnel and dried at room temperature from one experimental session to the next (Figure 1).

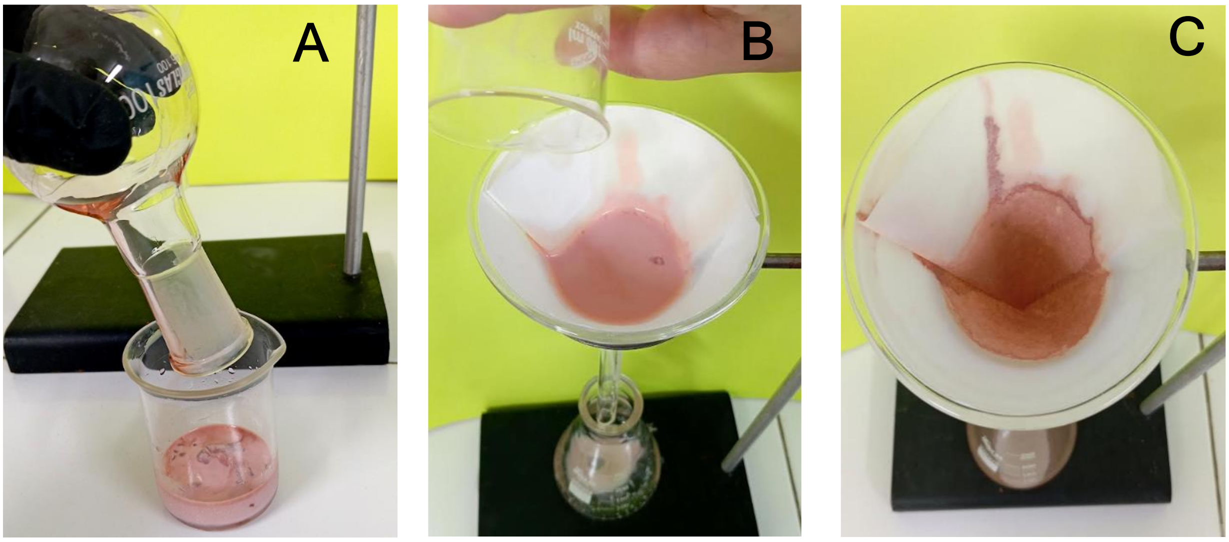


Fig.1. Recrystallization and filtration steps of the perimidine **3**. (A) Addition of a solution of crude product in ethanol to ice water. (B) Simple filtration step. (C) The product dried at room temperature after one day.

At the beginning of the second session, the students weighed the products obtained and were able to develop their knowledge of stoichiometric calculations to calculate the reaction yields. The yields of product **3** for the groups that used water as solvent were 76 and 85 %, while the yields found for the groups that used glycerol were 75 and 80%. The students realized that the use of glycerol as a reaction medium was more satisfactory for this educational experiment because of the solubility as mentioned above, and also by the possibility of several hydrogen interactions of the OHs of glycerol with the starting materials and possible intermediates, facilitating the condensation reaction [26].

Still in the second session, titled “characterization”, the students performed TLC and melting point analyses to confirm the purity of the product and spectroscopy techniques, such as UV-Vis, IR, and NMR to characterize the perimidine **3**. By TLC analyses (Figure S1) of the starting materials and product, the students applied their knowledge about the polarity and intermolecular interactions, where it was possible to observe that 1,8-diaminonaphthalene **2** presented a lower retention factor (Rf) than product **3** and benzaldehyde **1**, indicating that this diamine is more polar and has more interactions with the stationary phase of TLC. The melting points were determined by using a simple setup comprising a test tube, glycerol, a thermometer, and a Bunsen burner. The melting points of the products from each group presented values similar to the literature [27], and a range of temperatures of 106-108 °C, confirming the purity of the product and the successful synthesis.

As can be seen in Figure 1, the product has a characteristic coloration; therefore, a UV-Vis analysis is interesting for students to observe some electronic transition bands present in the product, such as π-π* transitions, since it is an aromatic compound, with a highly conjugated π electron system [29]. Furthermore, it was demonstrated to the students that from the UV-Vis spectra of product **3** at different concentrations, it is possible to determine the molar absorptivity coefficient (ε) from the Lambert-Beer equation (Equation S1), which can be used analytically in the future to determine the concentration of an unknown sample. The UV-Vis spectra of product **3** are shown in Figure 2 and the absorbance was plotted against perimidine concentration to determine the molar absorptivity at 233.5, 333.5, and 345.0 nm (Figure S2-S4 and Table S1).

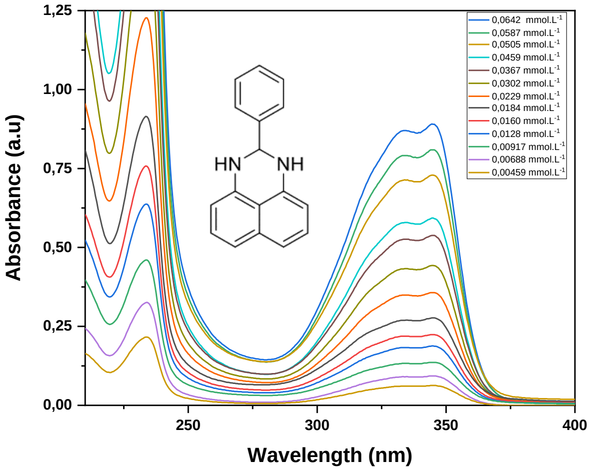


Fig.2. UV-Vis spectra of perimidine **3** at different concentrations in ethanol at 25 ºC.

Additionally, ^{1}H and ^{13}C NMR spectra, previously acquired, were used for pedagogical purposes. Thus, in the ^{1}H NMR analysis (Figure S5), it is possible to observe the characteristic signals of the formation of perimidine **3**, such as the CH group characteristic from the condensation reaction, the NHs of the amines, and all aromatic hydrogens. In the ^{13}C spectra (Figure S6), it is possible to verify the presence of the 17 carbons of the product, in a total of 11 signals, since the molecule presents symmetry and some chemically equivalent carbons.

Regarding the IR analysis (Figure S7), the comparison of the IR spectra of the product with the benzaldehyde can be discussed with the students to observe some characteristic bands that confirm the success of the reaction, such as the absence of the carbonyl stretching band (C=O) from the precursor aldehyde and the stretching bands of the NHs of the product.

The time allocated for each experimental step is detailed in Table S1. It is important to note that this experiment can be performed in any organic chemistry laboratory, containing simple and common equipment and glassware, and using relatively accessible and easy-to-handle reagents. In addition, the students were constantly evaluated during the experimental classes with an emphasis on their effective participation in the laboratory stages, and at the end of the class, they answered a questionnaire to discuss the main concepts involved in the experimental class, as a form of a report.

At the end of the two sessions, the same questionnaire distributed at the start of the experiment was applied again to measure students' progress. Furthermore, a satisfaction survey regarding the experiment was also conducted. Results from all questionnaires are available in the supporting information.

4. Conclusions

In this experiment, the undergraduate organic chemistry students successfully synthesized, quantified, and characterized 2-phenyl-2,3-dihydro-1*H*-perimidine using inexpensive and accessible reagents, suitable for any standard teaching laboratory. Through this hands-on activity, students applied theoretical knowledge to practice, mastering important concepts such as addition reactions, carbonyl reactivity, organic compound solubility, and imine formation. Additionally, they gained experience in essential laboratory techniques, including TLC monitoring, liquid-liquid extraction, and recrystallization.

Characterization techniques such as melting point determination, IR, UV-Vis, ^{1}H NMR, and ^{13}C NMR spectroscopy were introduced, providing students with valuable exposure to advanced analytical methods. The synthesis and purification were completed within a single session of four classes; however, this could be divided into two two-class sessions, offering flexibility for varied teaching environments.

Student feedback was overwhelmingly positive, indicating that the experiment was not only effective for learning but also engaging for participants. As such, this protocol represents a simple and efficient tool for teaching diverse organic chemistry concepts in higher education.

Supporting Information

Chemical hazards, details about the experiment, characterizations, questionnaires and practical class scripts are available.

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Author Contributions

Diego Borodiak Araújo: data curation, methodology, formal analysis. Cássio Siqueira: data curation, writing – original draft provide, methodology. Patrícia Appelt: data curation, visualization, resources. Nicoli Catholico: conceptualization, investigation, visualization. Giancarlo V. Botteselle: funding acquisition, supervision, writing – review & editing.

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