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Perfil físico-químico e compostos bioativos de extrato hidrossolúvel de amêndoa baru (*Dipteryx alata* Vogel)

Physical-chemical profile and bioactive compounds of water-soluble baru extract (*Dipteryx alata* Vogel)

Perfil físico y químico y compuestos bioactivos del extracto soluble en agua del almendra de barú (*Dipteryx alata* Vogel)

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Aline Torres da Silva
ORCID: https://orcid.org/0000-0002-5140-799x
Instituto Federal de Educação, Ciência e Tecnologia Goiano – Campus Rio Verde, Brasil
E-mail: alinetorresrosa@gmail.com
Felipe Torres da Silva
ORCID: https://orcid.org/0000-0001-6914-5169
Instituto Federal de Educação, Ciência e Tecnologia Goiano – Campus Rio Verde, Brasil
E-mail: feliperv16@hotmail.com
Jordana dos Santos Alves
ORCID: https://orcid.org/0000-0003-2725-2980
Instituto Federal de Educação, Ciência e Tecnologia Goiano – Campus Rio Verde, Brasil
E-mail: jordanadossantosalves@hotmail.com
Daiane Sousa Peres
ORCID: https://orcid.org/0000-0003-1272-034X
Instituto Federal de Educação, Ciência e Tecnologia Goiano – Campus Rio Verde, Brasil
E-mail: daianesousaperes@gmail.com
Lismaíra Gonçalves Caixeta Garcia
ORCID: https://orcid.org/0000-0002-8508-8982
Instituto Federal de Educação, Ciência e Tecnologia Goiano – Campus Rio Verde, Brasil
E-mail: lismairagarcia@hotmail.com
Priscila Alonso dos Santos
ORCID: https://orcid.org/0000-0003-1024-4343
Instituto Federal de Educação, Ciência e Tecnologia Goiano – Campus Rio Verde, Brasil
E-mail: priscila.santos@ifgoiano.edu.br

Resumo

Objetivou-se com este trabalho elaborar extrato hidrossolúvel à base de amêndoa do baru e avaliar a estabilidade física e química dos produtos resultantes, assim como o conteúdo de compostos bioativos. Foram elaboradas três formulações com diferentes proporções de baru:água mineral, sendo elas 1:6, 1:8 e 1:10. Foram utilizadas amêndoas do baru adquiridas no comércio local da cidade de Rio Verde, GO, Brasil. As amêndoas do baru (*Dipteryx alata* Vog.) foram higienizadas, sanitizadas, despeliculadas. Os extratos hidrossolúveis passaram pelos processos de trituração com água, centrifugação, pasteurização e envase. Sendo ao final identificados e refrigerados a 5ºC. Foram avaliados pH, acidez titulável, parâmetros de cor, atividade antioxidante e o teor de compostos fenólicos totais em todos os extratos hidrossolúveis. Quanto aos resultados, houve um decréscimo gradual do pH, a acidez variou para uma das formulações, havendo ainda uma intensificação na cor dos extratos. De acordo com a proposta do presente estudo a amostra extrato hidrossolúvel de baru na proporção (1:6) foi a que apresentou o melhor desempenho em relação às análises realizadas.

Palavras-chave: Compostos bioativos, Restrição alimentar, Estabilidade física.

Abstract

The objective of this work was to prepare a hydrosoluble base extract of baru nut and to evaluate the physical and chemical stability, as well as the content of bioactive compounds. Three formulations with different proportions of baru:mineral water were prepared: 1:6, 1:8 and 1:10. Baru almonds were purchased from the local market in Rio Verde, GO, Brazil. The extracts were prepared with water, centrifuged, pasteurized, packaged and refrigerated at 5ºC. The pH, titratable acidity, colour parameters, antioxidant activity and total phenolic content of all formulations. Satisfactory levels of the bioactive compounds were found in all the proposed formulations of water soluble extract of baru. The 1:6 formulation presented minor changes throughout the ten days of storage. The processing used in this study was useful for obtaining the water soluble extract of baru.

Keywords: Bioactive compounds, Food restriction, Physical stability.

Resumen

El objetivo de este trabajo fue elaborar extracto soluble en agua a base de almendra de barú y evaluar la estabilidad física y química de los productos resultantes, así como el contenido de compuestos bioactivos. Se prepararon tres formulaciones con diferentes proporciones de baru: agua mineral, siendo 1: 6, 1: 8 y 1:10. Se usaron almendra de barú compradas en negocios

locales en la ciudad de Río Verde, GO, Brasil. Las almendra de barú (*Dipteryx alata* Vog.) Fueron desinfectadas, desinfectadas, descascaradas. Los extractos solubles en agua pasaron por los procesos de molienda con agua, centrifugación, pasteurización y envasado. Finalmente identificado y enfriado a 5ºC. Se evaluó el pH, la acidez titulable, los parámetros de color, la actividad antioxidante y el contenido de compuestos fenólicos totales en todos los extractos solubles en agua. En cuanto a los resultados, hubo una disminución gradual del pH, la acidez varió para una de las formulaciones y también hubo una intensificación en el color de los extractos. Según la propuesta del presente estudio, la muestra de extracto de baru soluble en agua en la proporción (1: 6) fue la que presentó el mejor rendimiento en relación con los análisis realizados.

Palabras clave: Compuestos bioactivos, Restricción de alimentos, Estabilidad física.

1. Introduction

Barueiro (baru, *Dipteryx alata* Vogel) is a native species of the cerrado biome in Brazil. These fruit trees are found in the states of Mato Grosso, Mato Grosso do Sul, Goiás, Minas Gerais and the Federal District. Baru is used not only for its almonds but also for wood and vegetable oil extraction (Almeida, 1998; Pinto, 2001).

The determination of the bioactive compound composition and antioxidant capacity of native fruits of the cerrado add commercial and industrial value to these fruits. Moreover, this type of research contributes to the conservation of the cerrado biome and to the health of the population by reducing the harmful effects caused by free radicals (Oliveira et al., 2009). Food allergies can be defined as specific adverse reactions of the immune system to a food (Benhamou, Schappi Tempia, Belli, & Eigenmann, 2009). Lactose intolerance is characterized by a lack of the enzyme lactase in the small intestine (Cunha et al., 2008). Vegetal extracts can be substituted for milk; they are a viable alternative due to their nutritional values.

Beverages based on plant extracts (soybeans, rice, corn, chestnut, among others) are also called "vegetable milks". These beverages are recommended in cases of allergy to cow's milk protein and lactose intolerance (Oliveira, 2013). Water-soluble extract-based beverages of plant origin have commercial and nutritional appeal for their health-promoting aspects, such as the absence of animal fats and high levels of minerals (Carvalho et al., 2011). However, many other types of raw materials can be used to make beverages, such as almonds, nuts, rice, and oats (Wong, 2013).

In this context, the objective of the present work was to develop water-soluble baru extracts and to subsequently evaluate the physical and chemical stability of the resulting products. For each of the developed formulations of baru nut and water, the pH, acidity and colour were determined, and the presence of bioactive compounds was evaluated.

2. Materials and Methods

The present work is a quantitative study, which was carried out in the laboratory (preparation of the water-soluble extract, quantification of the compounds and data analysis) (Pereira et al., 2018).

2.1. Production of water-soluble almond extracts from baru

To prepare the water-soluble extracts, baru nuts were purchased from the local market in Rio Verde, GO; all the almonds were roasted, and the films were included. The methodology used for the production of water-soluble extracts from baru almonds was developed according to Cardarelli & Oliveira (2000) with some adaptations. The films were first removed from the almonds, and then the almonds were crushed. The grinding step was carried out using a blender with a power of 1400 rpm for 6 minutes with pasteurized mineral water. After grinding, the resulting cake (the product resulting from crushing the almonds with water) was sieved and then centrifuged at low speed for 30 seconds to remove possible residues. The resulting product (water-soluble extract) was packed in 150-mL transparent glass containers with a screw-type plastic cap. After packaging, the water-soluble extracts were pasteurized at 72 °C ± 2 °C for 20 minutes in a water bath. Then, the extracts were rapidly cooled and stored under refrigeration at 5 °C.

2.2. Experimental design

Three formulations of the water-soluble extract from baru almonds (EHB) were prepared by varying the ratio of baru almond:mineral water; these ratios were 1:6 (EHB1); 1:8 (EHB2) and 1:10 (EHB3). All of the formulations were processed in a similar manner; only the proportion of water used in each formulation was altered.

2.3. Physical and chemical stability of water-soluble baru almond extract

Every five days for 10 days, a sample was removed to determine pH, titratable acidity and colour. All analyses were performed in triplicate with two replicates.

2.3.1. pH and titratable acidity

pH was determined using a digital potentiometer (Bel Engineering® - W38). The apparatus was calibrated with buffer solutions of pH 4.0 and pH 7.0, and then the pH was directly read by immersing the electrode in a beaker, according to technique nº 981.12 proposed by the Association of Official Analytical Chemists (AOAC, 2010). The titratable total acidity was determined by titration with 0.01 N sodium hydroxide solution (NaOH) using 1% phenolphthalein, according to the AOAC (2010).

2.3.2. Colour

Colour determination was performed by quantifying the three parameters defined in the CIEL*a*b* system. The parameters L*, a* and b* were determined by the colorimeter (Hunterlab, Colour Flex EZ). L* defines the luminosity (L* = 0 black and L* = 100 white), and a* and b* define the chromaticity (chroma) (+a* = red and -a* = green; +b* = yellow and -b* = blue). The samples were placed in a 20 mm quartz cuvette and placed in the optical sensor, and the readings were performed. The chroma parameters (Equation 1) and the hue angle (Equation 2) were calculated with the determinations of the coordinates a* and b*.

$$C = \sqrt{a^{*2} + b^{*2}} \quad \text{(Equation 1)}$$

$$^{\circ}Hue = arc\ tg\ \left(\frac{b^*}{a^*}\right) \quad \text{(Equation 2)}$$

2.4. Analysis of bioactive compounds

2.4.1 In vitro antioxidant activity

Antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method according to Brand-William et al. (1995), with modifications according to Borguini & Torres (2009). Ethanolic solutions with different concentrations of the water-soluble extract

were prepared by adding 1000 µL of the DPPH radical (0.004% mv^{-1}), and the final volume was adjusted to 1200 µL with ethanol. After the reaction progressed for 30 minutes, the degree of discolouration of the DPPH radical, caused by the action of antioxidants, was measured in a spectrophotometer (TECNAL®) at 515 nm. The % discolouration calculations were carried out with Equation 3:

$$\% \text{ discolouration of DPPH} = \left[-1\left(\frac{\text{Abs of the sample - Abs of white}}{\text{Abs of the control}}\right)\right] * 100 \quad \text{Equation 3}$$

where, Abs is the absorbance of the sample, white Abs is the absorbance of the blank, and Abs control is the absorbance of the control (750 µL of methanol + 1.5 mL of DPPH).

The results as presented as IC50, i.e., the antioxidant concentration required to reduce the initial amount of free radicals by 50%; the values are reported as grams of sample per milligram of DPPH used in the reaction (Rufino et al., 2010). The analysis was performed in triplicate with two replicates.

2.4.2. Total phenolic content

The content of phenolic compounds was determined by spectrophotometry (TECNAL®) at 760 nm using the Folin-Ciocalteu reagent (Waterhouse, 2002). For the colorimetric reaction, an aliquot of 0.5 mL of a methanolic solution of the water-soluble extract was added to 2.5 mL of the Folin-Ciocalteu reagent and 2 mL of a 4% sodium carbonate solution. After two hours of incubation at room temperature sheltered from light, the absorbance was measured. The quantification was based on the establishment of the standard curve of gallic acid at the concentrations of 40, 80, 120, 160, 200, 240, 280, 320, and 400 $mg.L^{-1}$. The results are expressed as milligrams of gallic acid equivalent (EAG) per 100 grams of sample. The analysis was performed in triplicate with two replicates.

2.5. Statistical analysis of the data

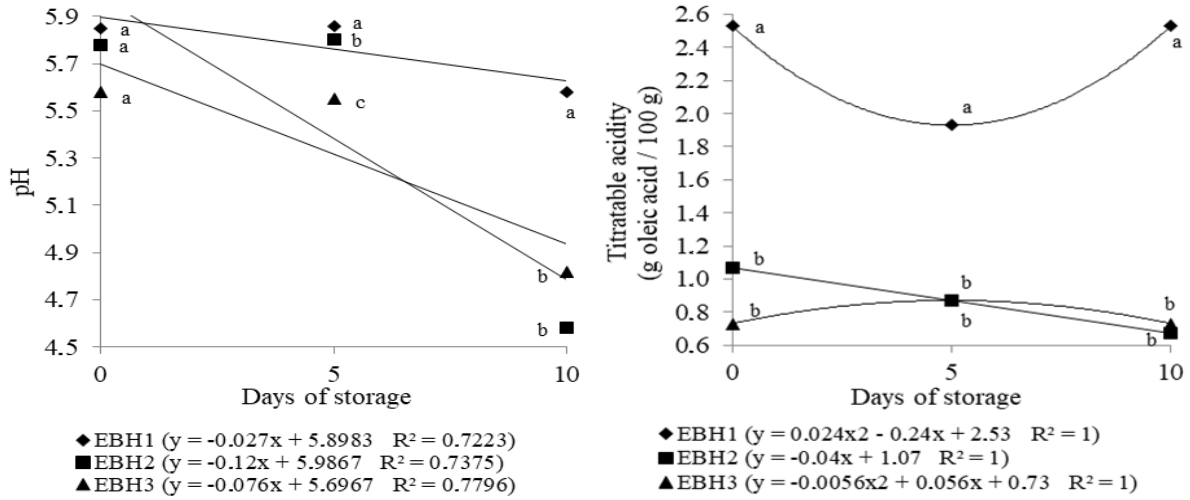
The pH, acidity, colour and bioactive compound data are presented as the mean ± standard deviation and were submitted to Tukey's test ($p > 0.05$) using ASSISTAT software version 7.7 (Silva, 2016).

3 Results and Discussion

3.1 Physico-chemical stability of water-soluble baru extracts

Figure 1A shows the pH and titratable acidity values of the water-soluble baru extracts over 10 days of storage under refrigeration at 5 °C.

Figure 1. Mean pH and titratable acidity of water-soluble baru extracts (*Dipteryx alata* Vogel) over 10 days of storage under refrigeration at 5 °C.



(A) (B)

Note: Means followed by the same lowercase letter at the same time (treatment comparison) were not significantly different from each other (p> 0.05) as determined by the Tukey test. EHB1, water-soluble baru extract (1:6); EHB2, water-soluble baru extract (1:8); EHB3, water-soluble baru extract (1:10). Source: Author (2019).

In this study, the water-soluble extracts maintained a pH between 4.58 and 5.86, as seen in Figure 1A, characterizing them as low acid foods. Thus, good manufacturing practices combined with food preservation methods that do not require the addition of preservatives are indispensable to provide product stability over a period of time.

Note that from day zero to day 10, there were significant variations in pH. Comparing the different formulations of the water-soluble baru extract, the EBH1 formulation (baru:water ratio of 1:6) had the highest pH values.

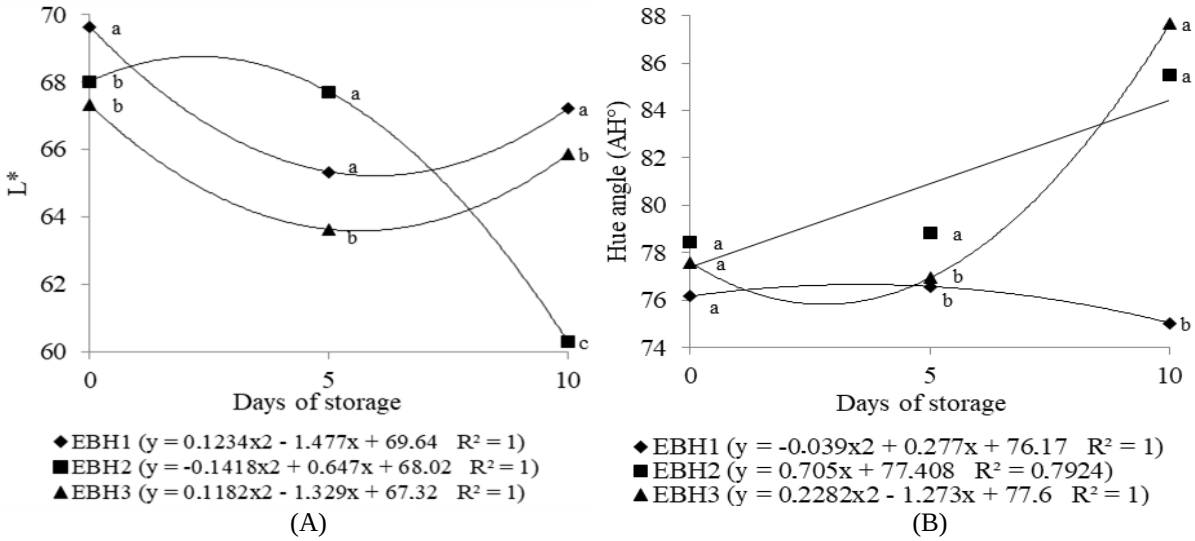
In the Santos study (2015), the performance of water-soluble extracts of Brazil nuts (*Bertholletia excelsa*) without preservatives was determined after thermal treatment pasteurization, packaging in polyethylene terephthalate (PET) bottles and storage under refrigeration at 5 ± 2 °C. It was observed that the variation in the pH values reported in the Santos study (2015) was similar to that in this study; a reduction in pH occurred over the storage period, and the extracts could be preserved for up to six days, with acceptable results.

In the study performed by Carneiro, Arévalo-Pinedo, Scartazzini, Giraldo-Zuniga, & Pinedo (2014), water-soluble extracts of babassu (*Orbignya speciosa*) were analysed following thermal treatment, as well as with the addition of pH stabilizers and preservatives, and the shelf life was extended by up to 60 days. Slightly acidic and preservative-free products, such as the water-soluble extract of baru, are more vulnerable to degradation, as they provide a favourable environment for fungi and bacteria, thus reducing their shelf life (Carneiro et al., 2014).

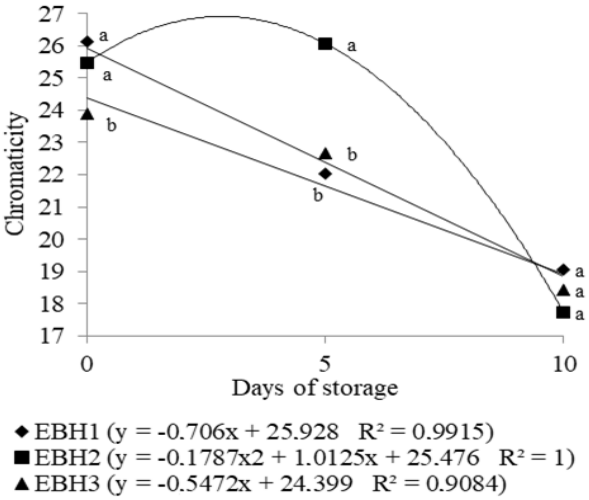
As shown in Figure 1B, there were significant variations in the acid values of the EHB1 formulation compared to those of the other treatments (EHB2 and EHD3) during storage. According to Chim, Zambiazi, & Rosane (2013), acidity is an important parameter of product quality; reactions involved in decomposition, such as hydrolysis, oxidation and fermentation, generate acidic compounds that consequently increase the acidity of the medium. At present, despite the variations observed with storage time, the acidity values of the three different formulations after 10 days of storage were equal or less than those at day zero (day of preparation).

Colour is another essential parameter for food quality, since a product with good sensorial quality is capable of fulfilling consumer expectations regarding characteristics such as appearance, taste, odour and texture (Cintra, 2015). Figure 2 shows the instrumental parameters of colour (L*, a* and b*) of the water-soluble baru extracts over 10 days of storage under refrigeration at 5 °C.

Figure 2. Mean colour parameter values (L*, hue angle and chromaticity) of the water-soluble baru extracts (*Dipteryx alata* Vogel) over 10 days of storage under refrigeration at 5 °C.



(A) (B)



(C)

Note: Means followed by the same lowercase letter at the same time (treatment comparison) were not significantly different from each other (p> 0.05) as determined by the Tukey test. EHB1, water-soluble baru extract (1:6); EHB2, water-soluble baru extract (1:8); EHB3, water-soluble baru extract (1:10). Source: Author (2019).

The L* (brightness) parameter varied from 0 (black) to 100 (white). On the tenth day of storage, all the treatments presented a statistically significant difference, as seen in Figure 2; however, all the treatments showed variations during storage, with values ranging from 60.31 to 6964, showing that all had a tendency to be a white colour. The values of the surveys performed by Carneiro et al. (2014), Santos (2015), Machado (2017) and Silva (2017) also show the same trend.

The hue angle calculations represent the colour attributes of red at 0°, yellow at 90°, green at 180° and blue at 270° (Souza, 2010). When considering the data of the present work, note that the hue angle values are close to 90 °C, corresponding to yellow colouration. Thus, the changes in the hue angle and luminosity parameters over time indicate that the product obtained a white-yellow colouration during the study period.

Chroma values express the intensity of the colour, that is, the saturation in terms of colour pigments. According to Mendonça, Jacomino, Mellhem, & Kluge (2003), chroma values close to zero represent neutral (greyish) colours, and values close to 60 represent vivid and intense colours. As shown in Figure 2C, all the treatments presented values closer to zero with reduction of the long of the storage. These results corroborate the luminosity and hue angle data.

3.2. Bioactive compounds of water-soluble baru extracts

Table 1 shows the antioxidant activity and total phenolic compound content of the water-soluble baru extracts.

Table 1. Mean antioxidant activity and total phenolic compound content of water-soluble baru extracts (*Dipteryx alata* Vogel) after 10 days of storage under refrigeration at 5 °C.

Samples	Antioxidant activity - IC_{50} ($\mu g.mL^{-1}$)*	Phenolic compounds (mg EAG.g^{-1})**
EHB1 (1:6)	30.50^{a}±1.37	96.33^{c}±4.23
EHB2 (1:8)	6.66^{b}±1.93	128.29^{b}±1.67
EHB3 (1:10)	3.49^{b}±0.11	277.70^{a}±2.10

Means followed by the same lowercase letter within a column were not significantly different from each other ($p > 0.05$) as determined by the Tukey test. EHB1, water-soluble baru extract (1:6); EHB2, water-soluble baru extract (1:8); EHB3, water-soluble baru extract (1:10). *Antioxidant concentration required to reduce the original content of free radicals by 50%. **Equivalents of gallic acid. Source: Author (2019)

The lower the IC50 value of a sample is, the higher its antioxidant activity because a smaller amount of the product is required to reduce the free radical DPPH content by 50%. Thus, it was observed that the EHB2 and EHB3 formulations presented the higher antioxidant activities than the EHB1 formulation, as seen in Table 1.

Roesler et al. (2007) evaluated the ability of the aqueous and ethanolic extracts of five cerrado fruits (araticum, lard, cagaita, lobeira and pequi) to sequester free radicals, and the lowest IC50 values were obtained with the aqueous extract of peanut bark (17.98 $\mu g.mL^{-1}$).

D'Oliveira (2015) observed a high antioxidant capacity of the water-soluble extract of the baru nut, as expected due to the dilution of bioactive compounds during processing; this effect was also confirmed in this study.

As observed in the antioxidant activity assay, the treatment with the highest content of phenolic compounds was EHB3, followed by EHB2. This result occurred because the protective effects of antioxidants of vegetable origin are closely related to the presence of phenolic compounds. When developing an aromatized baru drink, D'Oliveira (2015) reported a phenolic content value of 77.26 mg EAG.100 g $sample^{-1}$, which is lower than the phenolic content observed in the present study.

Lemos, Siqueira, Arruda, & Zambiazi (2012), in his study on the effects of roasting on the phenolic compounds and antioxidant capacity of baru almond, observed a significant reduction in the content of bioactive compounds as a result of the removing the coating film from the almond. Therefore, the use of baru almonds in the preparation of food formulations is recommended for their marked health-promoting aspects. However, as previously mentioned in the present study, all the almonds used were degummed before the formulations were prepared.

4. Conclusion

The processing method used in this study was useful for obtaining a water-soluble extract of baru. It is evident that the EHB1 formulation exhibited minor changes over the ten days of storage. Regarding the bioactive compounds, the EHB3 formulation was highlighted; however, despite the processing of these baru almonds, satisfactory levels of bioactive substances were found in all the prepared formulations of water-soluble baru extract.

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Porcentagem de contribuição de cada autor no manuscrito

Aline Torres da Silva – 20%

Felipe Torres da Silva – 16%

Jordana dos Santos Alves – 16%

Daiane Sousa Peres – 16%

Lismaíra Gonçalves Caixeta Garcia – 16%

Priscila Alonso dos Santos – 16%