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Research Article

Eugenia dysenterica extract microencapsulated for application in food

Extrato de Eugenia dysenterica DC. microencapsulado para aplicação em alimentos

Sebastião Moreira dos Santos Junior (ORCID 0009-0000-3680-5597), **Eliane Augusto Ndiaye** (ORCID 0000-0002-0321-5969), **Thiago Teixeira de Oliveira** (ORCID 0009-0005-5757-2303), **Keily Alves de Moura Oliveira** (ORCID 0000-0001-8974-8573), **Karina da Silva Chaves** *(ORCID 0000-0002-7454-898X)

Federal University of Mato Grosso, Barra do Garças, MT, Brazil. *Corresponding author: ksilchaves@gmail.com

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ABSTRACT

Eugenia dysenterica DC. is a fruit of the Cerrado that has great economic potential for both food and medicinal applications. Its phytochemical composition has potential health benefits, especially the phenolic compounds, which have significant antioxidant activity. However, being highly reactive, they can lose their stability and function under certain specific conditions, affecting their antioxidant potential. This study aimed to develop and characterize microparticles to protect the bioactive compounds present in the leaf of *E. dysenterica* DC. for application in food. The microparticles were produced using the ionic gelation technique with alginate and pectin (wall materials) and were assessed for morphology, average diameter, humidity, and encapsulation efficiency. The content of phenolic compounds and antioxidant activity was determined for the extract and microparticles. Qualitative analysis of the phenolic compounds and antioxidant activity was carried out by Thin Layer Chromatography (TLC). The microparticles were predominantly spherical in shape and had an average diameter of 46.06 ± 18.81µm, a moisture content of 92.01% and an encapsulation efficiency of 16.10%. When using methanol as the extracting solution, the leaf extract showed a total phenolic compound content of 171.89 mg EAG.100g^{-1} and antioxidant activity of 91.09%, while the microparticles showed a phenolic compound content of 27.65 mg EAG.100g^{-1} and antioxidant activity of 34.17%. Qualitative analysis indicated the presence of phenolic compounds and antioxidant activity in the extract, which was not detected in the microparticles. The low concentration of compounds and activity in the microparticles may be associated with the percentage of extract incorporated into the production process, extract concentration, and particle diameter. The concentration of the extract in the production process proves to be an important variable in improving the loading of phenolic compounds, since microencapsulation by ionic gelation has a potential application in foodstuffs as it provides protection for antioxidant compounds, while the size of the microparticles is an important factor in not altering the sensory perception of the food.

KEYWORDS: Cagaita. Microparticle. Ionic gelation. Cerrado.

RESUMO

Eugenia dysenterica DC., fruto do cerrado, possui grande potencial econômico de aplicação, tanto como alimento quanto medicinal. Sua composição fitoquímica exibe potencial efeito benéfico à saúde, destacando os compostos fenólicos, que possuem relevante atividade antioxidante. Porém, por serem altamente reativos, podem perder sua estabilidade e função em algumas condições específicas, afetando seu potencial antioxidante. Assim, o estudo visou desenvolver e caracterizar micropartículas na proteção dos compostos bioativos presentes na folha de *E. dysenterica* DC. para aplicação em alimentos. As micropartículas foram produzidas através da técnica de gelificação iônica utilizando alginato e pectina (materiais de parede) e avaliadas quanto a morfologia, diâmetro médio, umidade, eficiência de encapsulação. O teor de compostos fenólicos e atividade antioxidante foi determinado para o extrato e micropartículas. Análise qualitativa dos compostos fenólicos e atividade antioxidante foi realizada por

Cromatografia em Camada Delgada (CCD). A micropartícula apresentou formato predominantemente esférico e diâmetro médio de 46,06 ± 18,81µm, umidade de 92,01% e eficiência de encapsulação de 16, 10%. O extrato das folhas utilizando metanol como solução extratora, apresentou teor de compostos fenólicos totais de 171,89 mg EAG.100g^{-1} e atividade antioxidante de 91,09% e as micropartículas, um teor de compostos fenólicos de 27,65 mg EAG.100g^{-1} e atividade antioxidante de 34,17%. Análise qualitativa indicou presença de compostos fenólicos e atividade antioxidante no extrato e não foi detectado nas micropartículas. A baixa concentração de compostos e atividade nas micropartículas pode estar associado porcentagem de extrato incorporado no processo de produção, concentração do extrato e diâmetro da partícula. A concentração do extrato na produção mostra ser uma variável importante para melhorar o carreamento dos compostos fenólicos, uma vez que a microencapsulação por gelificação iônica apresenta potencial aplicação para alimentos por conferir proteção aos compostos antioxidantes e o tamanho das micropartículas, ser um fator importante para não alterar a percepção sensorial do alimento.
PALAVRAS-CHAVE: Cagaita. Micropartícula. Gelificação iônica. Cerrado.

INTRODUCTION

The Brazilian Cerrado exhibits great plant diversity and is an important source of income for the regional population. Some species have not yet been effectively studied, such as *Eugenia dysenterica* DC., popularly known as "cagaita" or "cagaiteira," a fruit-bearing tree native to the Cerrado biome (SILVA et al. 2001). It has a wide distribution, being found in several Brazilian states, such as Bahia, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Piauí, São Paulo, Tocantins and the Federal District (SILVA et al. 2015).

E. dysenterica DC. exhibits versatility in its potential applications, as different parts of the plant are popularly used both as food and in the treatment of various pathological conditions, such as skin treatment, intestinal disorders, microbial infections, inflammatory disorders, and *diabetes mellitus* (JORGE et al. 2010, LIMA et al. 2011, JUSTINO et al. 2022).

The benefits associated with the consumption of this plant are linked to the presence of antioxidant compounds, encompassing the classes of phenols, phenolic acids and their derivatives, flavonoids, tocopherols, terpenes, saponins, catechins, phospholipids, amino acids, phytic acid, ascorbic acid, pigments and sterols (ROESLER et al. 2007, JORGE et al. 2010, FERREIRA-NUNES et al. 2018, ARRUDA et al. 2022, JUSTINO et al. 2022).

However, these compounds are susceptible to degradation caused by factors such as pH, temperature, oxygen, light exposure, and storage conditions. Therefore, with the aim of providing protection to different compounds with bioactive properties, promoting their maintenance and bioavailability for a certain period of time, masking sensory characteristics, and allowing controlled release, different microencapsulation techniques have been applied in various industrial sectors (HOLKEM et al 2015, MELO & CONSTANI 2021).

The selection of the appropriate microencapsulation technique is based on the physicochemical properties of the material to be encapsulated and the encapsulating agent, taking into account the intended application of the food ingredient (VANISKI et al. 2017). Ionic gelation is a method that uses the electrochemical interaction characteristics of compounds, wall material, and bioactive substance, allowing different compounds to be incorporated into its structure, including solids, liquids, and

hydrophilic or hydrophobic substances (MELO & CONSTANT 2021). The process requires wall materials, such as carbohydrates, cellulose, lipids, proteins, or inorganic materials, in addition to a cross-linking solution, usually calcium ions, generating small droplets of the wall material and the compound, which will be encapsulated or coated (HERMAN-LARA et al. 2024).

In light of the above, this study aimed to develop ionically cross-linked microparticles, using alginate and pectin as wall materials for the protection of phenolic compounds present in the leaf extract of *E. dysenterica* DC., with a view to their application in food, and to determine the content of phenolic compounds and antioxidant activity of the extract and microparticles.

MATERIALS AND METHODS

Collection of *Eugenia dysenterica* DC.

The aerial parts of *E. dysenterica* DC. were collected between October and November on the premises of the Federal University of Mato Grosso, Araguaia University Campus, Unit I, in the municipality of Pontal do Araguaia. They were identified according to the herbarium specimen registered under number 09835, stored in the herbarium of the Araguaia University Campus. After collection and identification, the plant material was selected and subjected to drying at a temperature of 40 °C in a forced-air circulation oven (NOVA ETICA, 404/D, São Paulo, Brazil) for a period of 16 to 24 hours. Next, the material was ground in a Wilye-type knife mill (FORTINOX, STAR FT 80, São Paulo, Brazil) until a finely ground product with a powder-like appearance was obtained (LOPES 2017).

Obtaining the extract

The powder obtained after drying the leaves was subjected to the extraction process according to the methodology described by LOPES (2017) with modifications. A 400-gram sample of the material was placed in a light-protected flask, two liters of methanol were added, and the mixture was subjected to a maceration process for seven days with daily stirring to obtain the extract. After this period, the obtained extract was filtered and subjected to a concentration process in a rotary evaporator (FISATOM 801, São Paulo, Brazil) coupled to a vacuum pump (PRISMATEC, 131, São Paulo, Brazil) and condenser (SOLAB, SL 152, São Paulo, Brazil) maintained at a temperature of 50 °C. The concentrated extract was stored at -15 °C until use.

Production of microparticles

The microparticles were produced according to a methodology adapted from RIBEIRO et al. (2014). A dispersion of alginate:pectin (80/20) and 5% (v/v) of *E. dysenterica* DC. extract (21 $mg.mL^{-1}$) was sprayed onto a 2% (m/v) calcium chloride solution (pH 4.0) at room temperature, with a distance of 12 cm from the atomizer tip to the surface of the calcium chloride solution. During the atomization process, the solution was kept under constant stirring. After the atomization was complete, the solution was kept under constant stirring for 30 minutes to allow the microparticles to mature. The microparticles were then washed three times consecutively with distilled water (pH 4.0) using a 250 mesh sieve (BERTEL, São Paulo, Brazil) to remove excess calcium chloride, and stored under refrigeration until use.

Particle characterization

The particle was characterized in terms of moisture content (AOAC 2006) and average diameter. The average diameter of the microparticles was determined by image capture using an optical microscope (LEICA MICROSYSTEMS DM 750, Switzerland) at 40x magnification. For the determination, 200 images were collected, and the diameter of the microparticles was obtained individually, in duplicate, using the Laz Ez program, and the average diameter was calculated using Microsoft Excel® (MUKAI-CORREA et al. 2007, CARVALHO et al. 2019).

Encapsulation efficiency

The encapsulation efficiency was determined according to the amount of encapsulated material (ARRIOLA et al. 2019), according to the following equation:

$$EE\ (\%) = \frac{L}{Lo}\ x\ 100$$

Where: L = Total phenolic compounds in the sodium citrate solution of ruptured alginate and pectin microparticles; Lo = Total phenolic compounds in the *E. dysenterica* DC. extract.

Qualitative determination by Thin Layer Chromatography (TLC)

Phenolic compounds

The presence of phenolic compounds in the extract (21 mg.mL^{-1}) and in the microparticle was analyzed by TLC, using gallic acid as a positive control standard. The plate was eluted in $CHCl_3$/MeOH (9:1) and $CHCl_3$/MeOH/H_2O (65:30:5) and, after drying, the TLC plate was placed in a chamber containing iodine crystals, and the appearance of reddish-brown spots was observed, suggesting the presence of phenolic compounds, in comparison with the standard (SOUSA et al. 2007).

Antioxidant activity

The antioxidant activity of the extract (21 mg.mL^{-1}) and the microparticle was analyzed by CCD using gallic acid as a positive control standard. The plate was eluted in $CHCl_3$/MeOH (9:1) and $CHCl_3$/MeOH/H_2O (65:30:5) and, after drying, was sprayed with a 40 µg.mL-1 solution of the DPPH radical in ethyl alcohol. The plate was observed until the appearance of a yellow spot against a purple background, indicative of possible antioxidant activity (SOUSA et al. 2007).

Quantitative analysis of extract and microparticles

Phenolic compounds - Folin-Ciocalteau method

The phenolic compounds in the extract and microparticles were determined using the Folin-Ciocalteau method, with modifications (DELADINO et al. 2008). Before quantification, the microparticles were disrupted to extract the compounds according to the methodology described by ARRIOLA et al. (2019). A 2 mL aliquot of sodium carbonate, 200 µL of Folin-Ciocalteau reagent, and 200 µL of the solution (extract or microparticles) were added to a test tube, homogenized, and left to stand for 30 minutes protected from light. A standard curve of gallic acid with concentrations ranging from 10 µg.mL^{-1} to 512 µg.mL^{-1} was prepared. After this period, the reaction mixture containing the extract, the contents of the microparticles, and the points of the standard curve were subjected to reading in a spectrophotometer (FEMTO 600S, São Paulo, Brazil) at 750 nm. The total phenolic compounds were expressed in mg of gallic acid/100g of sample. The equation of the gallic acid calibration curve was Y = 0.0022x

+ 0.0945 and the correlation coefficient R = 0.9846. The analysis was performed in triplicate.

Antioxidant activity

The antioxidant analysis followed the methodology described by SOUSA et al. (2007) with modifications. The plant extract (21 mg.mL^{-1}) and the positive control (gallic acid) in ethanol were diluted to concentrations ranging from 10 µg.mL^{-1} to 512 µg.mL^{-1}, respectively. The microparticles were broken down to extract the compounds using 2% (w/v) sodium citrate (ARRIOLA et al. 2019). The absorbance of the reaction mixture, consisting of 0.3 mL of the solution (extract, microparticle, or positive control) and 2.7 mL of the DPPH stock solution at a concentration of 40 µg.mL^{-1}, was measured at 515 nm after 30 minutes. A mixture of ethanol (2.7 mL) and ethanolic solution of the extract (0.3 mL) was used as a blank. The calibration curve with DPPH was obtained at an absorbance spectrum of 515 nm. The equation for the DPPH calibration curve was Y = 0.0129x - 0.0082 and the correlation coefficient R = 0.9986. The analysis was performed in triplicate.

The percentage of antioxidant activity was calculated from the absorbance values at all tested concentrations, after 30 minutes, according to the following equation:

$$\%AA = \frac{\{[Abscontrol - (Absample - Abscontrol)x\ 100]\}}{Abscontrol}$$

Where: Abscontrol: initial absorbance of the ethanolic solution of DPPH;
Absample: absorbance of the reaction mixture (DPPH + sample).

Statistical analysis

Data analysis was performed using Microsoft Excel®. Results were expressed as mean ± standard deviation. Regression analysis was used to determine the phenolic compound content and antioxidant activity of the extract and microparticles.

RESULTS AND DISCUSSION

Characterization of particles

The alginate and pectin microparticles produced by the ionic gelation technique showed a moisture content of 92.01%, which is within the standards commonly found for microparticles produced by this technique (OTÁLORA et al. 2016). This result may be related to the high water absorption capacity of the biopolymers used as encapsulating materials, which are hydrophilic in nature (BELSCAK-CVITANOVIC et al. 2011).

Regarding morphology and size distribution, these showed varying diameters (Figure 1A) and a spherical shape with the plant extract distributed throughout the entire volume (Figure 1B). The average particle diameter was 46.06 ± 18.81 µm. Larger diameters were obtained by TOMÉ & SILVA (2022) using the same encapsulation technique for the entrapment of basil (*Ocimum basilicum*), parsley (*Petroselinum crispum*), rosemary (*Rosmarinus officinalis*), thyme (*Thymus vulgaris*), chervil (*Anthriscus cerefolium*) and chives (*Allium fistulosum*) extracts, with particle diameters ranging from 920.08 ± 11.63 to 754.28 ± 16.62 µm.

The variation in particle diameter when evaluated in different types of studies can

be associated with the type of atomizer, distance and diameter of the atomizer needle, and the level of the cationic solution and concentration of encapsulating solutions (MUKAI-CORREA et al. 2007).

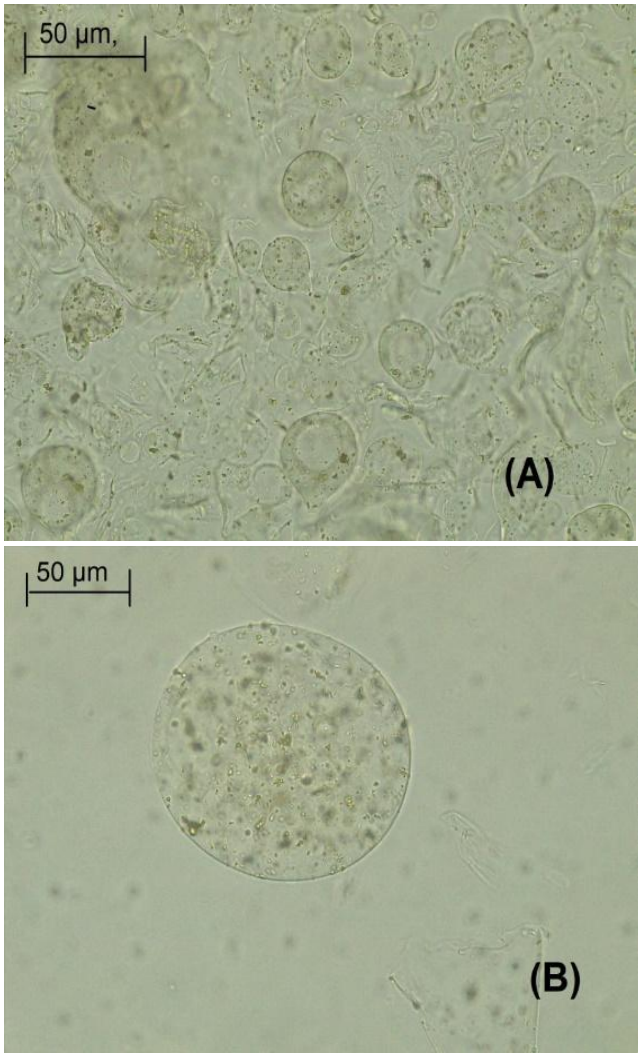

Figure 1. Optical microscopy of the morphology and size distribution of microparticles containing leaf extract of *E. dysentarica* DC (40x).

Microencapsulation efficiency

The encapsulation efficiency of the extract was 16.10%, a value considered low, indicating that the incorporation of 5% of the leaf extract at a concentration of 21 $mg.mL^{-1}$ was small, when compared to the values obtained in different studies, which showed encapsulation efficiencies of 45.00 to 93.39%, using the same microencapsulation technique (AZEVEDO & NORENÃ 2021, TOMÉ & SILVA 2022, BUDIN et al. 2023, ALEXANDRE et al. 2024). Factors such as the physicochemical characteristics of the active ingredient, the pH of the medium, the amount of active compound added to the formulation, the particle size, and the solubility of the coating with the active ingredient, as well as their interactions, can influence the encapsulation efficiency (SCHAFFAZICK et al. 2003, MARTINOVIĆ et al. 2023). Encapsulation efficiency ranging from 68.24 ± 0.15 to 93.39 ± 0.01% was observed in the production of microparticles with aromatic herbs using the ionic gelation technique and alginate as the wall material (TOMÉ & SILVA 2022). MARTINOVIĆ et al. (2023) describe that

better encapsulation efficiency of phenolic compounds can be achieved using proteins, since phenolic compounds and proteins can bind through hydrogen and hydrophobic bonds, and proteins can also be linked to free carboxyl groups of other coatings, such as sodium alginate.

Total phenolic compounds in the extract

The extract from the leaves of *E. dysenterica* DC. showed 171.89 mg EAG.100g^{-1}, a value higher than that found in the fruit, which was 111.00 mg EAG.100g^{-1} (ROCHA et al. 2013) and 143.81 mg EAG.g^{-1} (NASCIMENTO et al. 2020). ROCHA et al (2011) describe a difference in concentration between the green and ripe fruit of *E. dysenterica* DC., where 90.00 mg EGA.100g^{-1} and 111.00 mg EGA.100g^{-1} were observed, respectively. The results obtained in this study indicate that the leaves of *E. dysenterica* DC. are a good source of phenolic compounds. The importance of the extraction method and the type of solvent should be emphasized, as these can influence the extraction of phenolic compounds depending on their polarity (MOURA-FILHO et al. 2017, BORGES et al. 2022). The use of methanol in different concentrations has proven effective in extracting total phenolic compounds from various plants in the Cerrado biome, when compared to solvents such as ethanol, hexane, and water (SANTOS et al. 2016, MOURA-FILHO et al. 2017, BORGES et al. 2022).

Qualitative analysis of phenolic compounds and antioxidant activity

In the qualitative evaluation of phenolic compounds, reddish-brown coloration points were observed in the positive control (gallic acid – A) and in the plant extract (21 mg.mL^{-1} – B), suggesting the presence of phenolic compounds (Figure 2). These results corroborate the studies of BASTOS et al. (2016), who observed reddish-brown coloration points, indicating the presence of phenolic compounds in the extract of *Eugenia florida* DC.

However, the presence of these compounds was not observed in the microparticle after the rupture process (C) (Figure 2). The absence of these compounds may be associated with the amount of encapsulated extract, since a low encapsulation efficiency (16.10%) was observed. Factors such as particle size, concentration of the extract added in the microparticle production process, and low interaction of the extract with the polymers influence the encapsulation efficiency and, consequently, a reduction in the amount of biologically active compounds present (JYOTHI et al. 2010, MARTINOVIĆ et al. 2023).

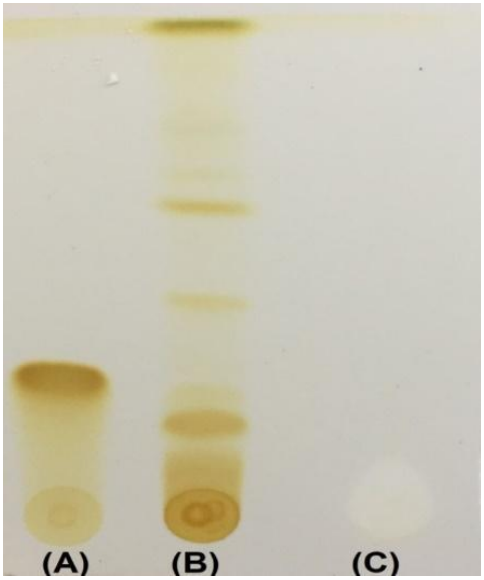

Figure 2. Qualitative evaluation of phenolic compounds by TLC with iodine elution.

Regarding antioxidant activity, the appearance of yellow spots against a purple background was observed in the positive control (gallic acid – A) and in the plant extract (21 $mg.mL^{-1}$ – B), indicating that the DPPH radical was consumed (Figure 3). Diverse points of yellow coloration appear in the elution of the extract (B), a positive characteristic for antioxidant activity (Figure 3).

In the microencapsulated extract (C), the appearance of the yellow coloration was not observed. The result obtained corroborates what was observed in the qualitative evaluation of phenolic compounds in the microparticles, since the antioxidant activity is directly related to the content of phenolic compounds. In most vegetables, phenolic compounds are the most abundant antioxidants (ROCHA et al. 2011). Therefore, the absence of yellow coloration points can be associated with the same factors described for the qualitative evaluation of phenolic compounds.

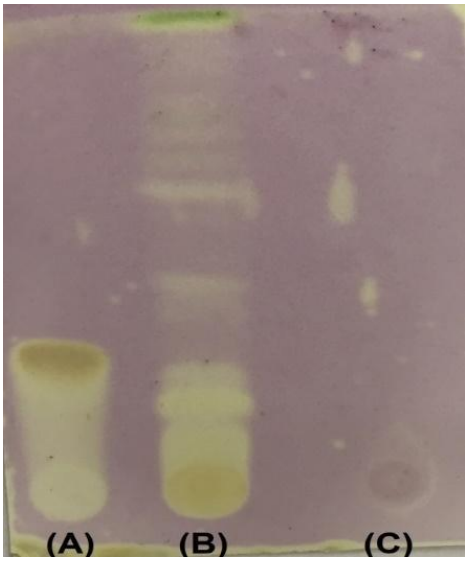

Figure 3. Qualitative evaluation of antioxidant activity by TLC eluting in ethanolic DPPH solution.

Quantitative assessment of antioxidant activity

The quantification of the antioxidant activity of the *E. dysentarica* DC. extract at a concentration of 21mg.mL^{-1} was 91.09%, a value higher than that found for the fruit pulp by SCHIASSI et al. (2020), which showed 73.57% antioxidant activity. Regarding the microencapsulated extract, the antioxidant activity was 34.17%. Ramírez et al. (2017) encapsulated the fruit extract by spray-drying and obtained an antioxidant activity ranging from 5.8 to 26.5%, lower than that observed in the present study. This shows that the ionic gelation technique, being a non-thermal method, allows for the maintenance of the stability of compounds with antioxidant activity. However, the antioxidant activity can be improved by increasing the encapsulation efficiency. Another factor that should be considered is that the interaction of a compound with antioxidant potential with DPPH depends on its structural conformation, where some compounds react very quickly with DPPH and reduce a number of DPPH molecules corresponding to the hydroxylated groups, while others require a longer reaction time for the consumption of DPPH (BRAND-WILLIAMS et al. 1995).

CONCLUSION

The extract of *E. dysenterica* leaves contains phenolic compounds with antioxidant potential, demonstrating the technological potential of this plant from the Cerrado biome for application in food. The encapsulation technique used proved to be quite viable for carrying these compounds and for application in food due to the small diameter, which is of utmost importance so as to not alter the sensory perception of the food. The concentration of the extract during production proved to be an important variable for improving the extraction of phenolic compounds present in the leaves of this plant commonly found in this biome. Future studies will be conducted to optimize encapsulation efficiency and target the application of the microparticles in food.

AUTHOR'S CONTRIBUTIONS

Conceptualization, methodology, and formal analysis, Sebastião Moreira dos Santos Junior, Eliane Augusto Ndiaye, Keily Alves de Moura Oliveira, and Karina da Silva Chaves; software and validation, Sebastião Moreira dos Santos Junior, Thiago Teixeira de Oliveira, and Karina da Silva Chaves; investigation, Sebastião Moreira dos Santos Junior, Eliane Augusto Ndiaye, Thiago Teixeira de Oliveira, Keily Alves de Moura Oliveira, and Karina da Silva Chaves; resources and data curation, Sebastião Moreira dos Santos Junior, Eliane Augusto Ndiaye, Thiago Teixeira de Oliveira, Keily Alves de Moura Oliveira, Karina da Silva Chaves; writing - original draft preparation, Sebastião Moreira dos Santos Junior, Thiago Teixeira de Oliveira, and Karina da Silva Chaves; writing - review and editing, Eliane Augusto Ndiaye, Keily Alves de Moura Oliveira, and Karina da Silva Chaves; visualization, Sebastião Moreira dos Santos Junior, Thiago Teixeira de Oliveira, and Karina da Silva Chaves; supervision, Eliane Augusto Ndiaye, Keily Alves de Moura Oliveira, and Karina da Silva Chaves; project administration, Eliane Augusto Ndiaye, Keily Alves de Moura Oliveira, and Karina da Silva Chaves; funding acquisition, Karina da Silva Chaves. All authors have read and agreed to the published version of the manuscript.

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STATEMENT OF THE INSTITUTIONAL REVIEW BOARD
Not applicable to studies that do not involve humans or animals.

INFORMED CONSENT STATEMENT
Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT
The data can be made available upon request.

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CONFLICTS OF INTEREST
The authors declare that there is no conflict of interest.

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