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# Phytochemical analysis of Cagaita (*Eugenia dysenterica DC*) leaf extracts in Northern Minas Gerais Cerrado, Brazil

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**Abstract**

The objective of this study was to perform a phytochemical screening of the aqueous extracts of the leaves of *Eugenia dysenterica* DC to identify the secondary compounds present; evidence the presence of total yellow flavonoid compounds; identify total phenolic compounds, evaluate antioxidant activity; and verify the influence of the different extraction methods on the concentration of the identified secondary metabolites. For phytochemical evaluation, three aqueous extracts were obtained using infusion, decoction, and maceration. The preparation followed the methodology of Garlet (2019), with adaptations. The phytochemical screening included the determination of the total phenolic content by the FolinCiocalteu method. Absorbance was measured spectrophotometrically. Antioxidant activity was determined by oxygen radical absorption capacity method described by Prieto et al. (1999). The determination of yellow flavonoids was performed as described by Francis (1982), with modifications. Maceration was the most effective method to extract total yellow flavonoids, while decoction was the most effective to extract total phenolic compounds. Maceration resulted in higher antioxidant activity. Statistical differences indicate that the choice of extraction method should be guided by the type of bioactive compound of interest and its thermal stability to maximize the extraction of certain secondary metabolites from *Eugenia Dysenterica* DC.

**Keywords:** *Eugenia dysenterica*; phytochemical screening; leaf extracts.

**Practical application:** The practical application of this study are extensive and promising, offering numerous opportunities for the development of natural products beneficial to human health. They can also guide future research and the selection of extraction techniques to maximize the achievement of specific bioactive compounds in *Eugenia dysenterica*, depending on the desired goal (total phenolics, flavonoids or antioxidant activity).

## 1 INTRODUCTION

*Eugenia dysenterica* DC, commonly known as cagaita, is a plant native to the Brazilian Cerrado, recognized for its medicinal and nutritional properties. This species, belonging to the Myrtaceae family, is widely used in traditional medicine to treat various conditions, such as diarrhea and intestinal disorders. In addition to its therapeutic applications, cagaita fruits are consumed *in natura* and used in the preparation of juices, liqueurs, and jellies.

Previous studies have investigated various properties of *Eugenia dysenterica*. Nunes (2018) developed, characterized, and evaluated nanoemulsions (NE) containing aqueous extract of *Eugenia dysenterica* leaves, including CD and catechin, as innovative therapeutic alternatives for delaying skin aging through antioxidant action. Another study was carried out by Prado (2013) to investigate the potential gastroprotective effects of aqueous extracts from the leaves of this plant. Karadag et al. (2009) assessed the *in vitro* antioxidant activity (DPPH) of plant extracts from the pulp, seed, and peel of the fruit of *Eugenia dysenterica.* However, in a search carried out in the Web of Science, PubMed, and Google Scholar databases with the keywords: "*Eugenia dysenterica*," "phytochemistry," and "extraction methods," revealed no studies, to date, that have performed a comparative quantitative phytochemical screening of aqueous extracts of *Eugenia dysenterica* leaves., for the identification of secondary compounds.

Therefore, the present study aimed to perform quantitative phytochemical screening of aqueous extracts of the leaves of

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*Eugenia dysenterica* DC, for the identification of secondary compounds obtained through different extraction methods. The specific objectives were to: evidence the presence of total yellow flavonoid compounds; identify total phenolic compounds; evaluate antioxidant activity; and verify the influence of various extraction methods on the concentration of the secondary metabolites identified.

In this context, this study is significant as it expands knowledge about the chemical composition of *Eugenia dysenterica* leaves, highlighting their potential applications in both the pharmaceutical and food industries, as a source of bioactive compounds with antioxidant properties.

## 2 MATERIALS AND METHODS

### *2.1 Obtaining the plant material*

The leaves of *Eugenia dysenterica DC* were collected in the afternoon at the Institute of Agrarian Sciences, located on the campus of the Center for Administrative and Didactic Activities (CAAD), a unit of Universidade Federal de Minas Gerais, in Montes Claros, Minas Gerais (MG), and the coordinates defined through Google Maps (16.685656, -43.846617) in July 2023.

### *2.2 Preparation of extracts*

For the quantitative phytochemical evaluation, three aqueous extracts were obtained using different forms of preparation: infusion, decoction, and maceration. The preparation procedures followed Garlet (2019), with adaptations. For the infusion, 2 g of plant material were weighed, and 200 mL of boiling water (≅ 100 °C) was poured over it. The mixture was covered and allowed resting for 10 minutes. For decoction, 2 g of fresh plant material were placed in 200 mL of distilled water and boiled for 10 minutes. In the maceration process, 2 g of leaves were manually crushed for 15 minutes in 100 mL of distilled water. Subsequently, the contents were then transferred to a beaker covered with aluminum foil, 100 mL of distilled water was added, and, finally, the mixture was left to stand at room temperature (25 °C) for 24 hours.

### *2.3 Analysis of total phenolic compounds*

The total phenolic content was determined by the Folin-Ciocalteau method, as described by Whaterhouse (2002), with some modifications. The FolinCiocalteau method is a colorimetric test that measures the antioxidant capacity of phenolic compounds. The sample (0.5 mL) of each aqueous extract (infusion/decoction and maceration) was homogenized with 2.5 mL of FolinCiocalteau 10% reagent (v/v). Then, 2 mL of the 4% (w/v) sodium carbonate solution was added. The tubes were shaken again for 1 minute and the reagent mixture was kept at rest for 2 hours in the dark. Absorbance was measured spectrophotometrically (UV-VIS SP-2000UV spectrophotometer) at 720 nm. A calibration curve was prepared using a solution of gallic acid (5–40 μg). Results were expressed as μg of gallic acid equivalent per mL of sample (μg GAE/mL). Analyses were performed in triplicate.

### *2.4 Analysis of antioxidant activity*

Antioxidant activity was determined using the phosphomolybdenum complex protocol, following the modified methodology by Prieto et al. (1999). This method is based on the reduction of $Mo^{6+}$, distinguished by its green color at acidic conditions, with maximum absorption at 695 nm. This method is processed at high temperatures and for a prolonged time, having the advantage of evaluating the antioxidant capacity of both lipophilic components and hydrophilic compounds in an acidic medium. A 0.1 mL aliquot of the sample (infused, neckline, and macerated extracts) was mixed with 3 mL of reagent solution (sulfuric acid 0.6 M, sodium phosphate 28 mM, and ammonium molybdate 4 mM). The tubes were capped and incubated in a water bath at 95°C for 90 min.

After the samples were cooled to room temperature (25°C), the absorbance of the green phosphomolybdenum complex was measured at 695 nm. A blank sample was made by placing 0.1 mL of water with 3 mL of the phosphomolybdenum complex. Quantification was based on a standard ascorbic acid curve (7.81 to 500 μg), and the results were expressed in mg of ascorbic acid equivalents (AAS) per mL of sample. The analyses were performed in triplicate.

### *2.5 Determination of yellow flavonoids*

The yellow flavonoid content was determined following the method described by Francis (1982), with modifications. Briefly, a 2.5 mL sample of each aqueous extract (infusion, decoction, and maceration) was mixed with 20 mL of an acidified ethanol solution (95% ethanol: HCl 1.5 N — 85:15 v/v). The samples were homogenized for 1 min and then transferred to a 50 mL volumetric flask. The volume was completed to 50 mL with the same ethanolic solution and incubated for 16 hours under refrigeration (7°C) without incidence of light. After this period, the extracts were filtered and the absorbances measured at 374 nm. The yellow flavonoid content was calculated using Equation 1, with an absorption coefficient of 76.6 (mol/cm). The analyses were performed in triplicate.

$$\text{Yellow flavonoid content (mg/100 mL)} = \frac{(\text{ABS} \times \text{dilution factor}) \times 10^3}{(\text{Sample volume} \times \varepsilon^{1\%}_{1\,\text{cm},374})} \quad (1)$$

Where:

ABS: the absorbance reading of the sample;

$\varepsilon^{1\%}_{1cm,374}$ : the absorption coefficient for yellow flavonoids.

### *2.6 Statistical analysis*

This experiment was based on a completely randomized design with three independent replications. A one-way analysis of variance (ANOVA) was performed to compare all variables, and Tukey's test was used to calculate significant differences at $p \leq 0.05$. Data analysis was performed using the Statistica software, version 10.0, and results were expressed as mean ± standard error.

## 3 RESULTS AND DISCUSSIONS

### *3.1 Assay of total yellow flavonoids*

The determination of yellow flavonoids was performed as described by Francis (1982), with modifications. As previously reported, the total dosage was calculated using Equation 1, and the results, expressed in mg/100 mL of fresh leaves, are shown in Table 1.

Table 1 shows the total yellow flavonoid content in different extracts of fresh leaves of *Eugenia dysenterica* DC, measured in mg/100 mL. The extracts were prepared using three methods: infusion, maceration, and decoction. The analysis of total yellow flavonoids in these extracts revealed that: for the infusion method, no total yellow flavonoids (ND) were detected in the analyzed samples. Regarding maceration, the total yellow flavonoid contents in the three samples were 43.081, 27.502, and 30.200 mg/100 mL, with a mean of 33.594 mg/100 mL and a standard deviation of 8.326. For Decoction, the total yellow flavonoid contents in the three samples were 10.360, 12.790 and 10.440 mg/100 mL, with a mean of 11.197 mg/100 mL and a standard deviation of 1.38.

The non-detection of total yellow flavonoids may indicate that the infusion method, as performed, is not effective to extract these compounds from the fresh leaves of *Eugenia dysenterica* DC. This may be attributed to the lower temperature and shorter extraction time, as noted by Hamed et al. (2019), who noted that efficient flavonoid extraction often requires methods that use higher temperatures and/or longer extraction durations.

The highest mean total yellow flavonoids (33.594 mg/100 mL) observed in maceration indicates that this method is highly effective for extracting these compounds. The relatively high standard deviation (8.326) suggests some variation between samples, but the concentration of flavonoids remains significant.

The high content of yellow flavonoids (YF) in maceration (33.59 ± 4.81 mg/100 mL) compared to decoction (11.20 ± 0.80 mg/100 mL) is supported by Zanusso et al. (2023), who reported maceration as particularly effective due to prolonged solvent contact without thermal degradation of the compounds.

The decoction method yielded lower levels of total yellow flavonoids compared to maceration, with an average of 11.197 mg/100 mL. The low standard deviation (1.38) indicates minimal variation between samples, suggesting that while the method is consistent, it is less effective than maceration.

### *3.2 Total phenolic compound analysis*

Table 2 shows the assay of total phenolic compounds in different extracts of fresh leaves of *Eugenia dysenterica DC*, measured in mg/100mL. The extracts were prepared by infusion, maceration, and decoction.

The data shown in Table 2 demonstrate that the total phenolic compound content extracted from the fresh leaves of *Eugenia dysenterica* DC varies significantly according to the extraction method used. Infusion had the lowest mean total phenolic compound content (2.3529 mg/100 mL) among the three methods tested. Standard deviation (0.7086) is relatively low, indicating consistency across samples. Maceration resulted in a significantly higher mean total phenolic compound content of 70.0829 mg/100 mL. Standard deviation (3.2576) indicates moderate variation between samples, reflecting good overall consistency.

Decoction produced the highest mean total phenolic compound content (142.3107 mg/100 mL). Although the standard deviation (9.9976) is higher compared to the other methods, indicating greater variability among samples, the concentration of phenolic compounds remains substantial. These findings highlight the efficiency of different extraction methods in isolating phenolic compounds, which is relevant for applications in pharmaceutical and nutraceutical industries. The statistically significant differences ($p \leq 0.05$) indicate the impact of the extraction technique on the phenolic compound yield.

Tomborelli et al. (2018) reported significant phenolic content in aqueous extracts of *Eugenia dysenterica* leaves, which corroborates the results found for decoction and maceration in this study.

The higher total phenolic content (TPC) result found in decoction is consistent with the findings by Soares et al. (2016), who noted that techniques involving prolonged heat, such as decoction, are more effective for phenolic extraction. Corroborating the results found in this study, decoction extraction appeared in some studies as the technique that obtains the best results in the extraction of phenolic content in different plant species (Oliveira et al., 2016). However, no previous studies have been found comparing infusion, maceration, and decoction techniques to extract biotic compounds from aqueous extracts of *Eugenia dysenterica* DC.

Infusion had the lowest TPC (2.35 ± 0.41 mg GAE/100 mL), which can be explained by the lower temperature and shorter extraction time since the extraction of phenolic compounds is less efficient at lower temperatures due to the heat helping to break the cell walls of the plants and release less of these compounds

**Table 1**. Total yellow flavonoid content in different extracts of fresh leaves of *Eugenia dysenterica* DC (mg/100 mL).

| Samples | Infusion | Maceration | Decoction |
|---|---|---|---|
| **1** | ND | 43.081 | 10.360 |
| **2** | ND | 27.502 | 12.790 |
| **3** | ND | 30.200 | 10.440 |
| **Mean** | | 33.594 | 11.197 |
| **Standard deviation** | | 8.326 | 1.380 |

**Table 2**. Assay of total phenolic compounds in different extracts of fresh leaves of *Eugenia dysenterica* DC (mg/100 mL).

| Samples | Infusion | Maceration | Decoction |
|---|---|---|---|
| 1 | 2.9900 | 66.4242 | 153.8545 |
| 2 | 2.4790 | 71.1553 | 136.6334 |
| 3 | 1.5896 | 72.6692 | 136.4441 |
| Mean | 2.3529 | 70.0829 | 142.3107 |
| Standard deviation | 0.7086 | 3.2576 | 9.9976 |

into the solution. According to Sete da Cruz et al. (2022), at a lower temperature, this breakdown is less effective, resulting in a lower extraction of these compounds. Similar results were observed by Veber et al. (2015), who also reported lower phenolic content in infusions of Jambolan leaves (*Syzygium cumini* L.).

### *3.3 Antioxidant activity*

Antioxidant activity was determined by the phosphomolybdenum complex protocol, according to the modified methodology described by Prieto et al. (1999). Quantification was based on a standard AAS curve (7.81 to 500 µg), and the results, presented in Table 3, were expressed in mg of AAS per 100 mL of sample.

Table 3 shows that the total antioxidant activity (TAA) extracted from fresh leaves of *Eugenia dysenterica* DC varies significantly according to the extraction method used. Infusion had the lowest mean AAT (14.10 mg/100 mL) among the three methods tested. Standard deviation (3.15 mg/100 mL) is relatively low, indicating consistency across samples.

Maceration exhibited the highest average TAA (1,760.147 mg/100 mL). Although standard deviation (253.484 mg/100 mL) is higher, indicating greater variability between samples, the antioxidant activity remains substantial. Decoction, in turn, produced an intermediate mean TAA (700.963 mg/100 mL), significantly higher than infusion but lower than maceration. Standard deviation (198.940 mg/100 mL) indicates considerable variation between samples.

These results suggest that maceration is not only efficient in the extraction of yellow flavonoids, but also in the extraction of other antioxidant compounds, such as polyphenols, anthocyanins, carotenoids, which contribute to AAT (Souza et al., 2021).

Statistically significant difference indicates that maceration may be preferable to maximize antioxidant activity, possibly due to the longer extraction time and the absence of high temperatures that may degrade some antioxidant compounds.

**Table 3**. Evaluation of total antioxidant activity in different extracts of fresh leaves of *Eugenia dysenterica* DC in mg of ascorbic acid equivalent per 100 mL of sample.

| Samples | Infusion | Maceration | Decoction |
|---|---|---|---|
| 1 | 12.370 | 2,049.040 | 591.700 |
| 2 | 17.740 | 1,656.440 | 930.590 |
| 3 | 12.190 | 1,574.960 | 580.590 |
| Mean | 14.100 | 1,760.147 | 700.963 |
| Standard deviation | 3.150 | 253.484 | 198.940 |

The higher TAA found in maceration is consistent with the literature. Oliveira et al. (2016) reported maceration often yields extracts with high antioxidant activity due to its effectiveness in extracting a wide range of antioxidant compounds.

Decoction showed significant TAA (700.960 ± 114.860 mg GAE/100 mL), although lower than that obtained via maceration. This outcome supports the findings of Zanusso et al. (2023), who noted that although decoction effectively extracts phenolic compounds, prolonged heat exposure may degrade some heat-sensitive antioxidants. Similarly, a study by Santos (2023) demonstrated that decoction is the most efficient method for extracting phenolic compounds from guava leaves. For Magalhães and Santos (2021), the higher efficiency of decoction in extracting phenolic compounds may be related to the combined effects of heating and the use of water as a solvent.

Infusion, with TAA of 14.100 ± 1.820 mg GAE/100 mL, showed the lowest antioxidant activity, corroborating the observations of Souza et al. (2019), who reported lower antioxidant capacity in *Eugenia dysenterica* infusions due to limited extraction of bioactive compounds compared to other methods.

### *3.4 Extraction methods at the concentration of secondary metabolites*

Table 4 presents the biotic compounds of aqueous extracts of *Eugenia Dysenterica* DC obtained by three different extraction techniques: infusion, maceration, and decoction. The objective is to verify how each extraction method influences the concentration of the identified secondary metabolites. The parameters analyzed were TPC, YF and TAA. The following results are presented, analyzed, and discussed in detail.

The results shown in Table 4 demonstrate that each extraction method has a different impact on the concentration of biotic compounds. Decoction resulted in the highest concentration of total phenolics (142.31 ± 5.77$^{a}$ mg GAE/100 mL), followed by maceration (70.08 ± 1.88$^{b}$ mg GAE/100 mL) and, finally, infusion (2.35 ± 0.41$^{c}$ mg GAE/100 mL). The different letters indicate statistically significant differences, with decoction showing a significantly higher concentration of total phenolics ($p \leq 0.05$).

The increase in TPC with decoction can be explained by the prolonged boiling involved in this method, which can break down plant cell walls more effectively, releasing more phenolic compounds. Gonçalves et al. (2013) showed that decoction often results in higher concentrations of total phenolics compared to other extraction methods due to its

**Table 4**. Bioactive compounds of aqueous extracts of *Eugenia dysenterica* obtained by three different techniques.

| Parameter | Extraction technique | | |
|---|---|---|---|
| | Infusion | Maceration | Decoction |
| TPC (mg GAE/100 mL) | 2.35 ± 0.41$^{c}$ | 70.08 ± 1.88$^{b}$ | 142.31 ± 5.77$^{a}$ |
| YF (mg/100 mL) | ND | 33.59 ± 4.81$^{a}$ | 11.20 ± 0.80$^{b}$ |
| TAA (mg EAA/100 mL) | 14.10 ± 1.82$^{c}$ | 1,760.15 ± 146.35$^{a}$ | 700.96 ± 114.86$^{b}$ |

Means ± standard error on the same line followed by different lowercase letters indicates statistically significant differences for $p \leq 0.05$, according to Tukey's test ($n = 3$); TPC: total phenolic content; GAE: gallic acid equivalent; YF: yellow flavonoids; EAA: equivalent in ascorbic acid; TAA: total antioxidant activity; ND: Not Detected.

greater ability to break through plant matrices and release bound compounds. Other studies examining the extraction of phenolic compounds from leaves of different plant species have observed that decoction extracted more total phenolics than infusion and maceration, confirming that high temperatures can be beneficial for the release of phenolic compounds (Figueirinha et al., 2008; Fotakis et al., 2016; Pérez et al., 2014; Ramalho et al., 2013).

Maceration showed the highest concentration of YF (33.59 ± 4.81[a] mg/100 mL), while the infusion did not detect any compound of this type. Decoction, though containing YF (11.20 ± 0.80[b] mg/100 mL), has a significantly lower concentration than maceration. This finding is corroborated by studies suggesting that maceration, which occurs at room temperature or with slight heating, better preserves thermolabile compounds such as flavonoids (Pinelo et al., 2005). Decoction may lead to partial degradation of heat-sensitive flavonoids, explaining the lower concentration observed.

Maceration stands out with the highest concentration of total antioxidant equivalents (1,760.15 ± 146.35[a] mg EAA/100 mL), followed by decoction (700.96 ± 114.86[b] mg EAA/100 mL) and, finally, infusion (14.10 ± 1.82[c] mg EAA/100 mL). The differences are statistically significant, with maceration showing a significantly higher concentration ($p \leq 0.05$). This result is in line with studies showing that maceration is effective in extracting antioxidants, especially when performed for prolonged periods that allow for a more complete extraction of soluble compounds (Ghafoor et al., 2009). Decoction, while effective, can degrade some antioxidants due to the high temperatures involved.

## 4 CONCLUSIONS

The results obtained in this study indicated that the presence of total YF compounds varies significantly according to the extraction method used. Maceration was the most effective method to extract total YF from fresh leaves of *Eugenia dysenterica* DC, while infusion was ineffective and decoction showed intermediate efficacy. These results can guide future research and practical applications aimed at extracting these bioactive compounds.

The analysis of the most efficient extraction method to obtain a higher concentration of TPC, demonstrated that the amount of these compounds extracted from the fresh leaves of *Eugenia dysenterica* DC varies significantly depending on the extraction method used. Decoction was the most effective method to extract total phenolic compounds, followed by maceration and finally infusion.

Maceration was the most effective method to obtain the highest antioxidant activity, followed by decoction and, finally, infusion. These results are important in determining which extraction method is most efficient to achieve greater antioxidant activity, which can be useful for functional food, cosmetic, and pharmaceutical applications.

The results presented for *Eugênia dysenterica* DC suggest that decoction is more effective for extracting TPC due to the efficient breakdown of cell matrices, while maceration is superior for the extraction of YF and total antioxidants, which can be degraded by high temperatures. Infusion, on the other hand, was less effective for all measured parameters. The statistically significant differences indicate that the choice of extraction method should be guided by the type of bioactive compound of interest and its thermal stability characteristics. This choice is crucial to maximize the achievement of certain secondary metabolites from *Eugênia Dysenterica* DC.

No other studies were found with the objective of performing a phytochemical screening of the aqueous extracts of *Eugenia Dysenterica* leaves to identify the secondary compounds present using decoction, maceration, and infusion extraction methods. However, the results obtained in this study are consistent with those that evaluated one of the extraction techniques and highlight the importance of choosing the extraction method to maximize the obtaining of bioactive compounds from *Eugenia Dysenterica* leaves.

For future research, it is recommended to specifically identify the phenolic and flavonoid compounds present in the extracts, as well as to evaluate the stability of these bioactive compounds over time and under different storage conditions. In addition, comparative studies with other extraction methods, such as ultrasound or microwave, could provide valuable insights into optimizing the extraction of bioactive compounds from *Eugenia Dysenterica.*

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