ORIGINAL ARTICLE

Evaluation of the potential of leaves and pods of *Libidibia ferrea* (Mart. ex Tul.) L.P.Queiroz as an antioxidant food supplement in the animal diet

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ABSTRACT

Libidibia ferrea (Mart. ex Tul.) L.P. Queiroz, commonly known as "*jucá*", is a plant of the Fabaceae family, widely distributed in northeastern Brazil. In folk medicine, it is used as an antioxidant, antidiabetic and anti-inflammatory. These effects are mainly attributed to its high content of phenolic compounds and flavonoids. Despite its known use in humans, the use of this plant as an antioxidant supplement for animals remains little explored. This study aimed to compare the levels of phenolic compounds and flavonoids in extracts of leaves and pods of *L. ferrea,* associating them with the potential for scavenging free radicals, as well as quantifying antinutritional factors. The levels of phenols and flavonoids were quantified by spectrophotometric and the scavenging potential was determined through DPPH radical assay. *L. ferrea* extracts showed antioxidant activity comparable to vitamin E, with leaf extracts at 50 µg/ml and pod extracts at 10 µg/ml, due to their high phenolic and flavonoid content. The recommended safe intake for animals is up to 115 g of leaf extract and 150 g of pod extract per day. These results suggest the potential of *jucá* extracts as an antioxidant supplement in animal feed, which may contribute to improving animal health and the quality of derived products.

Keywords: Oxidative stress, phytochemicals, Toxicity, Fabaceae, Animal supplementation.

INTRODUCTION

Libidibia ferrea (Mart. ex Tul.) L.P.Queiroz is a Fabaceae, popularly known as *jucá*. It is common in the northeast region of Brazil and is traditionally used to treat diabetes, asthma and gastrointestinal disorders (Da Costa et al. 2015; Hassan et al. 2015). The leaves of *L. ferrea* have antimicrobial and larvicidal activity (Da Costa et al. 2015). Experiments with Wistar rats using hydroethanolic extract with subsequent lyophilization and resuspension in saline solution, which were administered orally and intraperitoneally, indicated analgesic, anti-inflammatory and antiulcer activities for extracts of the fruits and stems of the species (Bachi and Sertié 1994; Carvalho et al. 1996).

Among the constituents that may be associated with these activities are polyphenols, secondary metabolites abundant in plants that include a wide variety of compounds, such as phenolic acids, lignans, stilbenes and flavonoids (Lin et al. 2016). These compounds are directly related to plant defense mechanisms and are currently being researched for use as antioxidants and in functional foods for animals such as cattle and goats to improve the quality of milk and its derivatives (Serra et al. 2021; Avila-Nava et al. 2023).

There are few studies on the nutritional potential of *jucá* for human and animal use. This underutilization of food resources from *jucá* can be attributed, in part, to the lack of scientific research exploring its nutritional value and its potential benefits for animal feed. However, preliminary studies indicate that the nutritional analysis of *jucá* leaf extract revealed a notable protein content (4.19%) and elevated levels of iron (21.65 mg/100 g) and zinc (31.52 mg/100 g), making it a particularly

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nutrient-dense option. These findings suggest that *jucá* may offer unique benefits for animal feed, as it combines essential proteins and minerals that support animal health (Chagas Neto 2018).

Caution is needed regarding antinutritional factors, compounds that, when consumed, reduce the nutritional value of foods. These compounds interfere with digestibility, absorption, or utilization of nutrients and, in high concentrations, can negatively impact health. Antinutritional factors may significantly reduce the bioavailability of essential amino acids and minerals (Benevides et al. 2011).

This research aims to investigate the potential of *L. ferrea* leaves and pods as a sustainable source of antioxidants for animal feed, focusing on livestock such as cattle and goats. Given its adaptability to arid and semi-arid regions, where the species is naturally abundant, *L. ferrea* offers a promising alternative for animal nutrition. By examining its safety, nutritional viability, and economic feasibility, this study seeks to position *L. ferrea* as a practical solution that could support sustainable agriculture, reduce feed costs, and enhance the resilience of animal husbandry in resource-limited areas.

MATERIAL AND METHODS

Plant material

The leaves and pods of *L. ferrea* were collected in the municipality of Caucaia-CE, Brazil. The identification was performed at the Prisco Bezerra Herbarium – Federal University of Ceará, where exsicata was incorporated under number 58443. This specie was registered in the Brazilian National System of Genetic Resource Management and Associated Traditional Knowledge (SisGen) with registration code A0C462C.

Production of *Libidibia ferrea* extract

After drying in an oven with air circulation at 38 °C, the leaves and pods (with the seeds) were pulverized separately in a multiprocessor until a powder was obtained. The materials were then sieved to a size of 1 mm. The extract of the leaves and pods was obtained by the maceration technique for 24 h, using 70% ethanol as the extracting solvent. At the end, 500 µg/ml extracts were obtained.

Determination of total phenol content

The analysis was performed using the Singleton and Rossi (1965) methodology. 100 µl of the sample was added to 0.5 ml of 1 N Folin-Ciocalteau reagent. Then, 1.25 ml of 20% saturated Na_2CO_3 solution was also added to this reaction. Finally, the solution had its volume adjusted to 10 ml with distilled water. After incubation for 40 min at room temperature and protected from light, the absorbance of the solution was measured at 785 nm in a spectrophotometer Genesys 10S. The total phenol content was determined using a calibration curve constructed with gallic acid (4 to 16 µg/ml). The result was expressed as mg GAE (gallic acid equivalents) per gram of herbal drug.

Determination of total flavonoid contents

The total flavonoid content was determined by the method of Ordon; Gomez and Vattuone (2006). Aliquot of 1 ml of the stock solution was used and added to 400 µl of the aluminum chloride solution, adjusting the volume to 10 ml with methanolic acetic acid solution. This solution was called the sample solution. The absorbance was measured at 425 nm, 30 min after the addition of the aluminum chloride solution. A solution containing alcohol, ethyl acetate, methanolic acetic acid solution and the displacement reagent were used as a blank.

The total flavonoid content was determined by interpolation of the sample substance against a calibration curve constructed with quercetin (0.5 to 24 µg/ml). The total flavonoid content was expressed in micrograms of quercetin equivalent (µg QE/ml).

Determination of nitrate levels

Nitrate quantification was determined according to the method described by Cataldo et al. (1975). For the analysis of *jucá* leaves and pods, 0.1 g of the respective samples were weighed and incubated in 10 ml of deionized water at a fixed temperature of 45 ºC, shaking every 15 min. After 1 h of incubation in a water bath, the samples were centrifuged at 2800 ×g for 15 min to sediment plant tissue residues. The supernatant was decanted into a test tube, removing 0.2 ml of each extract.

Next, 0.8 ml of salicylic acid solution in sulfuric acid was added and after 20 min, 19 ml of sodium hydroxide were added. The readings were taken in a spectrophotometer (410 nm). The sample tests were performed in triplicate and the results were expressed in µg NO_3-/100g of sample.

Determination of oxalic acid levels

The analysis was performed according to the AOAC methodology (1990) over four days. On the first day, 2 g of sample were weighed, 10 ml of 6 N HCl were added. One drop of caprylic alcohol and 20 ml of distilled water were added. After this step, the material was heated for 1 h in a water bath at 90 ºC with stirring. After cooling, the volume was completed to 50 ml in a volumetric flask. It was mixed and then left to stand overnight. On the second day, the material was filtered and 25 ml was pipetted into an Erlenmeyer flask. 5 ml of phototungstic acid was

added, after which the material was homogenized and left to stand for another night. On the third day, the material was filtered and 20 ml was pipetted into a centrifuge tube, ammonium hydroxide was added (drops) until pH 4.5 in the potentiometer. Aliquot of 5 ml of buffer solution was added, and after homogenization, it was left to rest in the refrigerator overnight.

On the last day, it was centrifuged for 20 min at 324 ×g. The precipitate was washed with ice-cold washing solution twice, after which the precipitate was dissolved in 10 ml of 20% sulfuric acid and transferred to an Erlenmeyer flask.

After that, it was titrated with 0.01 N $KMnO_4$ at a temperature of 60 to 80 ºC until the pink color persisted for 30 s. Each ml of 0.01 N $KMnO_4$ used in the titration corresponds to 0.4275 mg of anhydrous oxalic acid. The results were expressed in g/100 g of sample.

Antioxidant Activity: DPPH (Free Radical Scavenging Activity) assay

The antioxidant activity was determined according to the methodology of Saint-Cricq de Gaulejac et al. (1999). In a 96-well plate, each well with a volume of 300 µl. DPPH radical (292 µl) and 8 µl of the samples (leaves and pods of *jucá*) were used. The concentrations used in the samples were: 1, 10, 25, 50 125, and 250 µg/ml. 292 µl of the DPPH radical and 8 µl of 70% ethanol were used as a blank (control) for analysis and vitamin E at a concentration of 50 µg/ml was used as a standard. After 30 min protected from light, the reading was taken on a spectrophotometer at 517 nm. From the data found, it was possible to calculate the percentage of inhibition of the concentrations used according to the formula:

$$\% \text{ inhibition} = \frac{(\text{Control absorbance} - \text{Sample absorbance}) \times 100}{\text{Control absorbance}}$$

Statistical analysis

The results are expressed as mean ± standard error of the mean (S.E.M.) and the comparison between means was performed by one-way analysis of variance (ANOVA), followed by Bonferroni post hoc tests. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

The use of food supplements in raising animals, such as cattle and goats, is a common practice aimed at optimizing productive performance and ensuring the health and well-being of animals and improving the quality of their derived products (Ponnampalam et al. 2022). In this context, *jucá* (*L. ferrea*) emerges as a potential food supplement due to its nutritional composition and functional properties (Macedo et al. 2022). Although still little explored in this context, *jucá* presents promising characteristics that make it an attractive option to supplement the diet of these animals (Chagas Neto 2018).

Regarding the extracts of leaves and pods of *L. ferrea*, it was found that the extract of *jucá* pods presented a significantly higher content of phenolic compounds (230.57 mg GAE/g) compared to the leaves (47.10 mg GAE/g) (Table 1). Regarding the extracts of leaves and pods of L. ferrea, it was found that the extract of *jucá* pods had a significantly higher content of phenolic compounds (230.57 mg GAE/g) compared to the leaves (47.10 mg GAE/g) (Table 1). Comparatively, Port's and collaborators (2013) evaluated the total phenol content of nine species from the Amazon region, including *L. ferrea.* The authors obtained a content of 68.13 ± 15.92 mg GAE/g. Silva et al. (2011) analyzed three plant species for antioxidant capacity and DNA protection and observed that *L. ferrea* fruits had the ability to eliminate the damage to DNA. This antioxidant activity was associated with the high content of phenolic compounds of the species (460.00 ± 4.16 mg EAG/g). Prazeres et al (2019) found a higher value of total phenols in the pods of *L. ferrea* (951.39 ± 0.01 mg GAE/g) after preparation by the maceration technique for three days using 40% ethanol.

The discrepancy observed in the levels of phenolic compounds in different parts of the plant can be attributed to a variety of factors, including the location and time of collection, as well as the edaphoclimatic conditions, such as temperature and soil type, associated with the cultivation of the species. In addition, the differences can be influenced by the operational variables involved in the extraction process, such as the type of solvent and the extraction technique used. Furthermore, it is possible that this discrepancy is explained by the presence of other classes of phenolic substances, such as tannins, which tend to be in greater quantity in the pods compared to the leaves (Kobayashi et al. 2015).

Antioxidants play a protective role against oxidative damage, associated with several diseases that can be triggered by excess free radicals in the body, such as cancer, hypertension, heart disease, and diabetes (Chaudhary et al. 2023). Analyzing the results of the free radical scavenging antioxidant activity, it is possible to observe that the levels of phenolic compounds have a significant impact on the percentage of DPPH radical scavenging (Figure 1). It was observed that in relation to the extract of *L. ferrea* leaves, from the concentration of 10 µg/ml, the inhibition was already close to

Table 1. Total phenolic and flavonoid content in hydroalcoholic extract of *Libidibia ferrea* leaves and pods.

Hydroethanolic extract	Total phenols (mg GAE/g dry extract)	Flavonoids (mg QE/g dry extract)
Leaves	47.10±1.31	0.376±4.40
Pods	230.57±13.79	0,072±3.21

50% (IC_{50} = 50% radical inhibition concentration), agreeing with the research carried out by Hassan et al. (2015), who found IC_{50} = 12.45 ± 2.86 µg/ml for *jucá* leaves. However, these values were lower than the concentrations evaluated by Port's et al (2013), IC^{50} = 46.70 ± 4.01 µg/ml. Furthermore, from a concentration of 25 µg/ml (81.99%) for the leaves, the DPPH radical scavenging activity was comparable to that of vitamin E (94.32%). Regarding the pods, at an even lower concentration of 10 µg/ml (92.68%) there was radical scavenging comparable to the vitamin E reference standard (93.03%). The pod extract studied showed a superior DPPH radical scavenging capacity to that found by Prazeres et al (2019), who obtained a concentration of IC_{50} = 28.96 µg/ml. Although the extract of *L. ferrea* pods evaluated by Prazeres et al. (2019) has a higher content of total phenols compared to that studied in the present research, it was found that the scavenging potential was lower. This can be explained by the type of phenolic compounds extracted (Chaudhary et al. 2023).

The presence of phenolic compounds in the leaves and pods of *L. ferrea* is indicative of the antioxidant properties present in the species. Phenolic compounds are known for their ability to neutralize free radicals in the body, protecting cells against oxidative damage and reducing oxidative stress (Rahman et al. 2021). In dairy goats and cows, it was found that supplementation with phenolic compounds resulted in significant improvements in the antioxidant activity of milk. In addition, the addition of phenolic compounds such as hesperidin and naringin increased the antioxidant capacity of milk and reduced oxidative biomarkers, such as malondialdehyde. These compounds were also associated with metabolic benefits for those who consumed them, such as reduced body weight and fat mass, improved glucose tolerance, and prevention of liver complications (Avila-Nava et al. 2023).

In addition to evaluating the presence of antioxidant compounds such as polyphenols and flavonoids, the levels of the antinutritional compounds nitrate and oxalic acid were verified (Table 2). According to the European Food Safety Authority (EFSA), the maximum limit for nitrate intake is up to 64,000 µg/kg/day for adult cattle; available data suggest that sheep and goats are not more sensitive than cattle (EFSA 2020). Observing the results found in this research, it is assessed that, in relation to the nitrate content, the consumption of a dry extract of the leaves and pods would be safe for animal consumption.

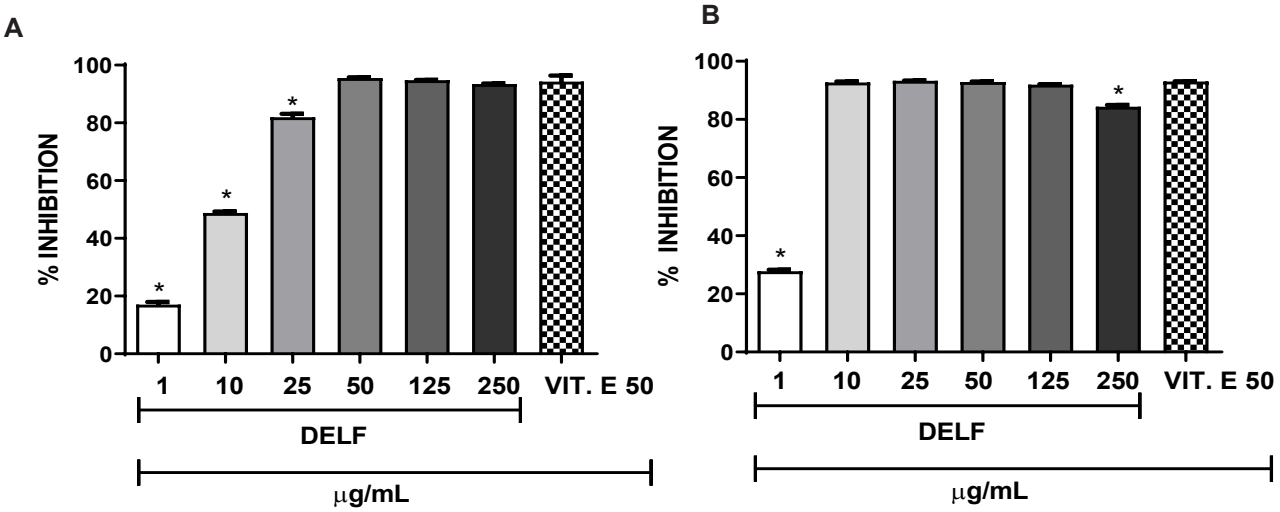

Figure 1. Antioxidant activity, DPPH test of hydroalcoholic extract of *Libidibia ferrea* leaves and pods. Percentage of inhibition of the DPPH radical by the leaves of the dry extract of *Libidibia ferrea* (A) and percentage of inhibition of the DPPH radical by the pods of the dry extract of *Libidibia ferrea* (B). Values represent mean ± SEM; *p<0.05 vs Vit. E; one-way ANOVA followed by Bonferroni post hoc test.

Table 2. Nitrate and oxalic acid content in hydroalcoholic extract of *Libidibia ferrea* leaves and pods.

Hydroethanolic extract	Oxalic acid (g/100 g dry extract)	Nitrates (μg NO_3^-/100 g dry extract)
Leaves	5.771	10.897
Pods	4.489	56.025

The oxalic acid content was also evaluated in the leaf and pod extracts of *L. ferrea.* Oxalic acid can bind to dietary calcium (Ca) or magnesium (Mg) to form insoluble Ca or Mg oxalate, which can lead to low serum Ca or Mg levels, as well as renal failure due to precipitation of these salts in the kidneys (Rahman et al. 2012). The induction of acute oxalate poisoning depends on several factors, including the chemical form of oxalate, the age of the animal, the rate of consumption, the quantity and quality of other foods consumed simultaneously, the total amount of oxalate consumed, and the adaptation to an oxalate-containing diet (Rahman et al. 2012). Rahman and collaborators (2017) evaluated goats weighing approximately 46 kg that doses higher than 0.146 g of oxalic acid/kg/day can affect the intake of dry matter, organic matter, crude protein, neutral detergent fiber and metabolizable energy, tending to decrease linearly with increasing oxalic acid administration. However, oxalic acid administration had no effect on the apparent digestibility of dry matter, organic matter, crude protein and neutral detergent fiber. This suggests that, although oxalic acid can influence feed and nutrient intake, it does not affect the digestibility of these nutrients in goats. Furthermore, non-ruminants appear to be more sensitive to oxalate than ruminants because in the latter, ruminal bacteria help to degrade oxalate. In this sense, observing the oxalic acid levels in the *L. ferrea* extract (5.77 g/100 g and 4.48 g/100 g for leaves and pods respectively), it is assessed that consumption should be moderate, not exceeding 115 g of leaf extract and 150 g of pod extract of *L. ferrea* per day.

Grisi and collaborators (2020) evaluated other antinutritional factors in the flour obtained from the pods and peels of *L. ferrea*. The authors quantified the levels of tannic acid, phytic acid, and oxalic acid. The results indicated a slightly higher tannic acid content (88.91 mg/100 g) compared to the peel (87.83 mg/100 g). Although tannins are known for their antinutritional effect by inhibiting the absorption of proteins and other nutrients, they also have significant antioxidant action. The presence of tannic acid, therefore, does not represent a limitation for the application of *jucá* flour in food products, especially due to its contribution to the antioxidant capacity of the bioactive compounds present.

Phytic acid levels were higher in fruit flour (3.86 mg/100 g) than in peel flour (1.80 mg/100 g). Phytic acid is known to chelate essential minerals such as iron and zinc, reducing their bioavailability. However, the impact of phytates can be mitigated depending on the food preparation method and the presence of other compounds. In addition, phytic acid also has antioxidant properties, which adds functional value to *jucá* flour. Finally, regarding the oxalic acid content, none of the flour samples presented detectable amounts of oxalic acid (Grisi et al. 2020).

CONCLUSION

This study highlights the noteworthy levels of phenolic compounds and flavonoids present in the extracts of leaves and pods of *L. ferrea*, which exhibit promising antioxidant activity. While the findings suggest the potential of these extracts as a nutraceutical option for improving animal health, it is important to note that further research is required to evaluate their safety and efficacy through toxicity testing. These results provide a foundation for future investigations into the application of *L. ferrea* extracts in animal nutrition, potentially enhancing the overall quality and health benefits of animal-derived products.

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AUTHOR'S CONTRIBUTION

Conceptualization: FCCN, LKAML, and PMPT; Methodology: FCCN; Formal analysis: LKAML and PMPT; Investigation: FCCN; Data curation: LKAML; Writing: FCCN; Original draft preparation: LKAML and PMPT; Writing - review and editing: LKAML and PMPT; Visualization: PMPT; Supervision: LKAML; Project administration: LKAML; Funding acquisition: LKAML. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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