

# Chromatographic and Spectrophotometric Analysis of Phenolic Compounds from Fruits of *Libidibia ferrea* Martius

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## ABSTRACT

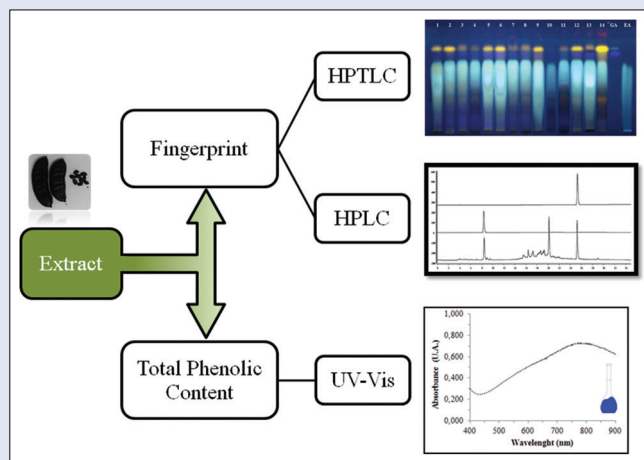
**Background:** *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz (*Fabaceae*) is a tree which is native to Brazil, widely known as “Jucá,” where its herbal derivatives are used in folk medicine with several therapeutic properties. The constituents, which have already been described in the fruit, are mainly hydrolysable tannins (gallic acid [GA] and ellagic acid [EA]). **Objective:** The aim of this study was to investigate the phenolic variability in the fruit of *L. ferrea* by ultraviolet/visible (UV/VIS) and chromatographic methods (high-performance liquid chromatography [HPLC]/high-performance thin layer chromatography [HPTLC]). **Materials and Methods:** Several samples were collected from different regions of Brazil and the qualitative (fingerprints by HPTLC and HPLC) and quantitative analysis (UV/VIS and HPLC) of polyphenols were performed. **Results:** The HPTLC and HPLC profiles allowed separation and identification of both major analytical markers: EA and GA. The chemical profiles were similar in a number of spots or peaks for the samples, but some differences could be observed in the intensity or area of the analytical markers for HPTLC or HPLC, respectively. Regarding the quantitative analysis, the polyphenolic content by UV/VIS ranged from 13.99 to 37.86 g% expressed as GA or from 10.75 to 29.09 g% expressed as EA. The contents of EA and GA by liquid chromatography-reversed phase (LC-RP) method ranged from 0.57 to 2.68 g% and from 0.54 to 3.23 g%, respectively. **Conclusion:** The chemical profiles obtained by HPTLC or HPLC, as well as the quantitative analysis by spectrophotometry or LC-RP method, were suitable for discrimination of each herbal sample and can be used as tools for the comparative analysis of the fruits from *L. ferrea*.

**Key words:** *Libidibia ferrea*, high-performance liquid chromatography, high-performance thin layer chromatography, phenolics compounds, spectrophotometry

## SUMMARY

- The polyphenols of fruits of *Libidibia ferrea* can be quantified by UV/VIS and HPLC
- The HPLC method was able to detect the gallic and ellagic acids in several samples of fruits of *Libidibia ferrea*

- The phenolic profiles of fruits from *Libidibia ferrea* by HPTLC and HPLC were reproducible.



**Abbreviations used:** HPTLC: high performance thin layer chromatography, HPLC: high performance liquid chromatography, UV-Vis: spectrophotometry

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## INTRODUCTION

*Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz is a tree native to Brazil, which belongs to the family *Fabaceae*,<sup>[1]</sup> it is widely distributed in the Northern and Northeastern regions,<sup>[2]</sup> where it is popularly known as “Pau ferro” or “Jucá.”<sup>[3,4]</sup>

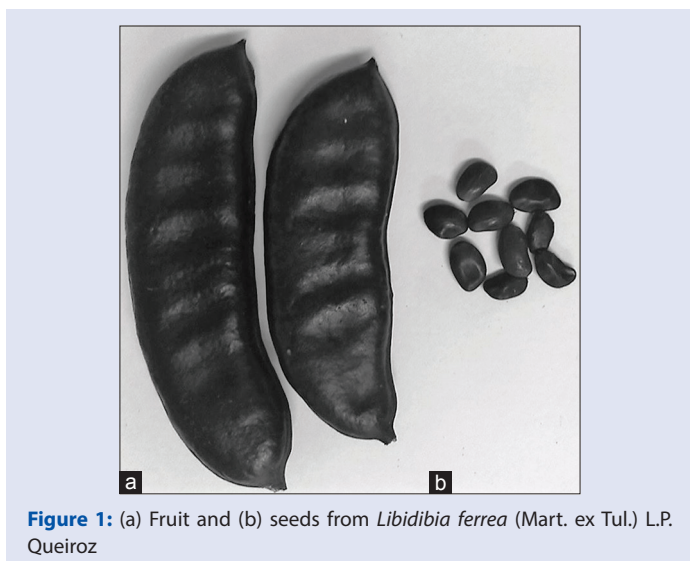
It has been reported that various parts of this species (bark, fruits, leaves, and seeds) are used in Brazilian folk medicine with several pharmacological properties. In this sense, several biological properties are reported for the fruits of *L. ferrea* [Figure 1]. The methanolic extract showed antifungal and antibacterial activities against oral pathogens;<sup>[5]</sup> and the aqueous crude extract showed related antiulcer, anti-inflammatory, and analgesic effects.<sup>[3,6]</sup> In addition, the aqueous infusion has been used by the population in the prevention of cancer.<sup>[7]</sup> Moreover, the aqueous extract of the fruit presented antiviral properties of sulfated polysaccharide for herpes simplex virus

type-1 and poliovirus type-1,<sup>[8]</sup> and extracts and polysaccharide fractions of pods demonstrated anti-inflammatory activity.<sup>[9]</sup> Several authors attribute the activities to the polyphenols and polysaccharides, which are the main constituents of the aqueous extracts.<sup>[9-11]</sup>

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**Figure 1:** (a) Fruit and (b) seeds from *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz

Phytochemical investigation revealed the presence of flavonoids, saponins, tannins, and other phenolic compounds in the hydroalcoholic extracts of stem bark, bark, and leaves.<sup>[12-14]</sup> Gallic acid (GA), methyl gallate, and ellagic acid (EA) were isolated from the fruit.<sup>[7,10]</sup> Polysaccharides were reported many times in the seed of *L. ferrea*.<sup>[8,9,11,15,16]</sup>

Based on this information, the hydrolysable tannins (gallotannins and ellagitannins) have the potential to be considered the main component of the fruit of this species, either by their concentration in the drug material or by their pharmacological properties attributed to this species. The total phenolic content is usually measured using the Folin-Ciocalteu spectrophotometric method, which is also adopted by the Brazilian Pharmacopoeia<sup>[17]</sup> and the European Pharmacopoeia.<sup>[18]</sup> However, possible interference has been described. To increase the specificity and eliminate interferences, several methods using high-performance liquid chromatography (HPLC) have been described for the determination of GA and<sup>[19-21]</sup> EA,<sup>[22-24]</sup> both in herbal drugs and biological matrices.<sup>[25]</sup> Once, GA and EA are determined in fruit and to prove the suitability of the method developed, it is necessary to perform validation of the methodology to establish acceptable parameters as well as to determine the total amount of the main compounds that are appropriate for the quality control.<sup>[26,27]</sup>

Furthermore, chromatographic techniques can be used to document the phytochemical fingerprints of chemical markers to identify variations in the herbal material. The analyses by thin-layer chromatography (TLC) play an important role as analytical tools for several codices such as the Chinese<sup>[28]</sup> and European<sup>[18]</sup> Pharmacopoeias. Recently, the use of high-performance TLC (HPTLC) improved the TLC technique due to its superior resolution and reproducibility.<sup>[29,30]</sup>

In this context, the present study was performed to develop and establish useful and simple procedures by HPTLC, HPLC, and spectrophotometry, which are able to detect, identify, and/or quantify the hydrolysable tannins and its variability in the drug material from the fruits of *L. ferrea*.

## MATERIALS AND METHODS

### Chemicals, reagents, and materials

Reference substances: GA (96% purity) and EA (from tree bark, 95% purity) were purchased from Sigma-Aldrich® (USA). Methanol was HPLC grade (J.T. Baker®, USA) and purified water was used (PURELAB® Classic UV, ELGA LabWater, USA). Other chemicals and solvents were of analytical grade.

### Plant material

The fruits of *L. ferrea* were collected in Limoeiro (Pernambuco, Brazil). The plant material was identified, a voucher specimen (herbarium specimen number 88145) was deposited at the Pernambuco Agronomic Institute (Instituto Agrônomico de Pernambuco - IPA), and this sample was used as a reference material. Another 13 samples were collected from different locations to verify the ability of the analytical procedures to evaluate the inter-sample variability.

### High-performance thin layer chromatography analysis

Each of the 14 samples was prepared by extracting 1 g of powdered material with 25 mL of methanol for 1 min in a water bath at 85°C. After that, the extracts were filtrated in cotton and 25 µL of each of the samples and standards were applied at 7 mm band width, in tracks 1-16 in the following sequence on plate: Herbal samples (1-14), GA (15), and EA (16). The HPTLC system (Camag®, Switzerland) consisted of a Linomat V sample applicator using 100 µL syringe (Hamilton®, Schweiz) connected to compressed air and winCATS® software (CAMAG®, Switzerland). Solvents for extraction and HPTLC grade solvents were purchased from J.T. Baker® (USA). Pre-coated TLC silica gel 60 F<sub>254</sub> aluminum plates (20 cm × 10 cm; 250 µm thickness; Merck®, Germany) were used. The plate was developed in a twin trough vertical glass chamber (20 cm × 10 cm; Camag®, Switzerland) using ethyl acetate:formic acid:water (90:5:5, v/v/v) as the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25 ± 2°C). After development, the plate was dried and the components were visualized by ultraviolet (UV) irradiation at 254 nm. Then, the plate was derivatized by spraying the NEU + PEG reagent and visualized under 365 nm. Each analysis was carried out in duplicate. The UV observations and image acquisitions were performed using MultiDoc-It Imaging System® (Model 125, USA) with UVP® software and a Canon® camera (Rebel T3, EOS 1100 D).

### Preparation of the extracts

#### Stock solution

The herbal drug extract was prepared using 1.0 g in a 250 mL round-bottomed flask with 150 mL of purified water and using the following procedure: Heat in a water-bath (LUCA-150/24/D; Lucadema®) for 30 min; cool under water and transfer quantitatively to a 250 mL volumetric flask; rinse the round-bottomed flask and collect the washings in the volumetric flask, and then diluted to 250 mL with purified water. After the suspended solids settle down, the solution was filtered through a filter paper and the first 50 mL of the filtrate was discarded.

### Determination of total phenolic content

An aliquot of 5 mL from stock solution was then transferred to a 25 mL volumetric flask and the volume was completed with purified water (S1). Subsequently, an aliquot of 2 mL from S1 was transferred to a 25 mL volumetric flask, 2 mL of Folin-Ciocalteu and 10 mL of water were added, and the volume was filled with a solution of anhydrous sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) 29%.<sup>[17,18]</sup> The samples were measured at 760 nm in an UV/visible (UV/Vis) spectrophotometer (Micronal®) at 30 min after the addition of the last reagent. Water was used as the blank. EA and GA were used as standards and results were expressed as equivalent per gram of dry weight of sample. Tests were carried out in triplicate.

### High-performance liquid chromatography analysis

#### Standard solution

Standard solution of EA was prepared by accurately Weighing 5.0 mg in a volumetric flask of 10 mL. Then, it was dissolved in dimethyl

sulfoxide (Vetec®) and placed in the ultrasonic bath for 30 min for the complete dissolution. From this solution, aliquots were transferred to a 10 mL volumetric flask and diluted with methanol:water (3:2, v/v). The standard solution of GA was prepared by accurately Weighing 25.0 mg in a volumetric flask of 25 mL, dissolving in purified water, and placing in the ultrasonic bath for 30 min for the complete dissolution. From this solution, aliquots were transferred to 10 mL volumetric flasks and diluted with purified water.

### Sample preparation

An aliquot of 3.75 mL from stock solution was then transferred to a 10 mL volumetric flask and the volume was completed with purified water. Then, this solution was filtered through a 0.45 µm polyvinylidene difluoride membrane (15 mm, Macherey-Nagel®) and stored in vials.

### Chromatographic conditions

Quantification of EA and GA was conducted on a HPLC (Ultimate 3000, Thermo Fisher Scientific®), coupled to a diode array detector (DAD; Thermo Fisher Scientific®) and equipped with a binary pump (HPG-3x00RS, Thermo Fisher Scientific®), a degasser, and an autosampler equipped with a loop of 20 µL (ACC-3000, Thermo Fisher Scientific®). For data analysis and processing, the software Chromeleon 6.8 (Dionex, Thermo Fisher Scientific®, USA) was used. The wavelength was set at 254 nm for detection of the EA and 270 nm for GA, in accordance with the maximum absorption measured by DAD. The chromatographic separations were achieved with a C<sub>18</sub> column (250 mm × 4.6 mm i.d., particle size 5 µm) from Dionex® equipped with a guard column (C<sub>18</sub>, 4 mm × 3.9 µm, Phenomenex®). Separations were carried out at a column oven temperature of 24°C. The mobile phase consisted of purified water (A) and methanol (B), both acidified with 0.05% trifluoroacetic acid (TFA), at a flow rate adjusted to 0.8 mL/min. A gradient program was applied as follows: 0–10 min, 12.5–25% B; 10–15 min, 25–40% B; 15–25 min, 40–75% B; 25–30 min, 75–75% B; 30–33 min, 75–12.5% B.

### Wavelength selection, specificity, and identification of the peaks

The wavelength for the determination of peaks was performed by scanning the standards, EA, and GA, in the range from 190 nm to 400 nm. The analysis of the spectrum was used to confirm the identity of each compound of interest. In addition, scans from 190 nm to 400 nm were performed for the extract solution from the herbal material and for the spiked extractive solution with the standards (EA and GA) to verify the occurrence of deviation from the maximum wavelength. Therefore, the peaks in the samples were identified by comparing their retention time and area with the standards.

### Validation procedure for quantitative analysis

The liquid chromatography (LC)-DAD procedure was validated in accordance with the guidelines from ICH (International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use – Q2/2005) and Brazilian Regulatory Agency – ANVISA (RE 899/2003).<sup>[26,27]</sup>

### Quantification of ellagic acid and gallic acid

The concentrations of EA and GA were obtained using the corresponding calibration curves.

## RESULTS AND DISCUSSION

### High-performance thin layer chromatography analysis

The chromatographic analyses suggest that the phenolics are probably the main compounds in the fruits of *L. ferrea*. Bands (spots) were characterized in this study under UV at 254 nm before spraying and

under 365 nm after spraying with NEU + PEG reagent. Thus, the R<sub>f</sub> values were calculated and compared with standards.

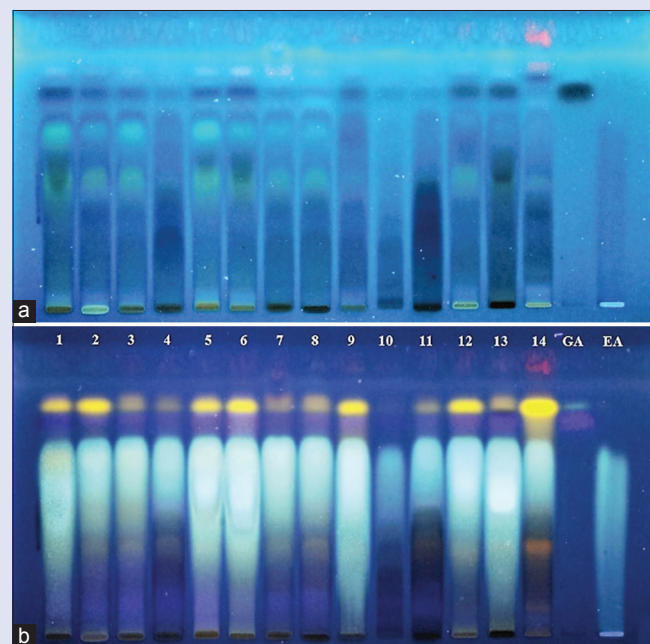
On the HPTLC plates, EA and GA were detectable as dark band under 365 nm before spraying with NEU + PEG reagent solution. After spraying with NEU + PEG reagent solution, the plates were analyzed and the EA showed a band with green color and the GA was blue. Derivatization with NEU followed PEG and visualization under 365 nm was best suited for band detection.

The chromatography profiles for the polyphenolic compound of all samples are shown in Figure 2. The samples showed bands corresponding to both the standards (EA and GA), confirmed by the color and R<sub>f</sub> (R<sub>f</sub> EA 0.60 and R<sub>f</sub> GA 0.68). The samples coded as 1, 5, 6, 12, and 13 showed higher intensity for the EA and GA spots. Furthermore, an additional yellow band at R<sub>f</sub> 0.73 was also detected in all samples. In addition, oranges bands typical for flavonoids at R<sub>f</sub> 0.31 and R<sub>f</sub> 0.10 could be observed in some samples. The first orange spot was observed in samples 8, 11, and 14; the second orange spot was detected only in the sample from “Brasília.”

Hence, the HPTLC analysis with semi-automatic applicator was able to separate and identify the compounds of interest. All samples showed positive results for both substances: EA and GA, confirming the relevance of such phenolic compounds for the drug material. Concerning the variability observed among the samples, it was expected and attributed to different conditions of plant development such as geographic origin (collection), climate, and soil conditions.<sup>[31,32]</sup> Thus, the procedure's ability to detect the differences was achieved and proved be able to support the establishment of standard fingerprint to assure the chemical and biological properties of such raw material and their extractives.

### Phenolics content

Regarding the relevance of phenolics compound on the biological properties of herbal materials, several studies have been conducted to determine the content of polyphenols either in plant material or crude



**Figure 2:** High-performance thin layer chromatography fingerprint of 14 samples of fruit from *Libidibia ferrea*. (a) Plate before de-derivatization. (b) Plate after derivatization with NEU + PEG reagent; 1–14: Samples; GA: Gallic acid; EA: Ellagic acid

extracts.<sup>[14,21,33-35]</sup> This class of compounds is present in the fruits of *L. ferrea* and it is related to several activities of this species.<sup>[9,11]</sup> The total phenolic content (TPC) of several samples from the fruits of *L. ferrea* were analyzed by UV/Vis and expressed by EA and GA [Table 1]. Higher values of TPC were observed for samples 1, 6, and 9, ranging from 24.96–37.85 g% to 18.02–27.33 g%, expressed as EA and GA, respectively. The samples 2, 5, 8, 10, 12, 13, and 14 showed intermediary values of TPC (about 20 g% of GA or 15 g% of EA) whereas the sample 4 showed the lower content of TPC (about 14 g% of GA or 11 g% of EA). The results of TPC showed similar behavior observed for the HPTLC analysis, where the intensity of the revealed bands was higher for the samples with higher TPC.

### High-performance liquid chromatography analysis

For the optimization of the chromatographic condition, preliminary analyses were carried out to find a good separation of the EA and GA compounds in a short possible total run time. Thus, several mobile phases and gradient elution programs were evaluated. The chromatographic separation was performed with an elution gradient system using water/methanol due to the wide polarity of the compounds. Several mobile phases were evaluated using different concentrations of water/methanol, with a flow rate of 0.8 mL/min and the gradient elution conditions selected using 12.5–25% of methanol, which is the separation of GA. However, in these conditions, EA did not elute, so this compound could only be eluted with a high concentration of methanol (75%).<sup>[25]</sup>

Both mobile phases were acidified with TFA to adjust the pH and prevent the ionization of the hydroxyl groups, and the separation was much more efficient after acidification. In this study, three different acids were tested: TFA, acetic acid, and phosphoric acid, and TFA at 0.05% proved to be the most effective to obtain relatively better resolution and separation. Hence, in this analysis, the presence of EA and GA standards were detected in large quantities, based on chromatographic profile, both were chosen as a markers for the fruit of *L. ferrea* [Figure 3].

### Wavelength selection, specificity, and identification of the peaks

The chromatograms were monitored at 254 nm for EA and 270 nm for GA, the maximum absorptions observed for both standards in the UV

spectrum. In these conditions, EA and GA were identified as the major compounds in the fruit from *L. ferrea*. EA and GA were well separated within 30 min and the retention times were 8.5 and 25.0 for GA and EA, respectively.

### Calibration curves, limit of detection, and limit of quantification

The calibration curves for EA and GA, using five concentration levels, were linear in the range from 23.2 to 34.9 µg/mL and from 24.0 to 36.0 µg/mL, respectively. For the herbal material, the linear range is from 1200 to 1800 µg/mL. The R<sup>2</sup> values were above 0.99 in all cases. Using the analyses of variance, the *F*-test for lack of fit was carried out for each calibration. As the *F*-statistics estimated by the ratio (lack of fit mean squares)/(pure error mean squares) were smaller than the *F*-distribution value with 4 and 10 degrees of freedom ( $\alpha = 0.05$ ), there was no evidence of lack of fit.<sup>[36]</sup> The results, summarized in Table 2, show that the method is sensitive enough to detect and quantify the EA and GA in the fruits of *L. ferrea*.

### Repeatability and intermediate precision

The reproducibility of procedure can be affected by many factors such as glassware, pipettes, and/or analysts.<sup>[38]</sup> In this context, the amounts of EA and GA were determined using fresh standard solutions and extractive solution on each day. For evaluation of the repeatability of the method, six independent samples (at 100% of the test concentration) were analyzed by HPLC. The content of EA and GA were 2.11 g% and 2.25 g%, with relative standard deviation (RSD) of 0.40% and 0.34%, respectively [Table 3]. The experiments for intermediate precision evaluation were carried out on two different days and the analyses were conducted by two different analysts, thus expressing the inter-laboratory variations. The results for quantification of EA showed RSD values of between 0.50% and 0.63%, and for quantification of GA between 0.27% and 1.05%, which are in accordance with RE 899/2003.<sup>[26]</sup>

### Accuracy

In the investigation of accuracy, known amounts of EA and GA, at different concentration levels (80, 100, and 120%), were added to the aqueous extract and the samples were then analyzed using the previously described method. Recoveries between 99.1% and 105.2% (RSD = 4.0%) were obtained for the EA and between 100.6% and 106.5% (RSD = 3.9%) for the GA. The results show that the developed method is satisfactorily accurate. According to the observed recovery rates, the method by LC-reversed phase-DAD applied to the fruit of *L. ferrea* presents a performance within the limits recommended by the Brazilian guide and related in literature, indicating that the analytical answers are mainly due to the analyte.<sup>[26,27]</sup>

### Robustness

The robustness should be evaluated during the development of the HPLC method by performing small changes in the original method, such as the pH of the mobile phase, flow rate, and column temperature.<sup>[37]</sup> Based on this, changes have been made in parameters critical to the process. The parameters that have been modified and the results are shown in Table 4. There were no significant differences in the area, retention time, and amounts of EA and GA, with RSD <5%. Thus, the methods for both EA and GA were considered robust under the conditions evaluated.

After the development and validation of HPLC method, the samples collected from the different regions were also submitted to the HPLC analyses, to verify the viability of methodology and similarities or differences with the authentic sample. The chromatographic profiles (fingerprint) are observed in Figure 4.

**Table 1:** Fourteen samples collected from different regions in Brazil, total phenolic content by ultraviolet/visible and content of tannin by high-performance liquid chromatography

Samples	TPC (g%) by UV/VIS		Content g% by HPLC	
	GA	EA	GA (270 nm)	EA (254 nm)
1. Limoeiro (region 1) - PE	32.86 (0.21)	25.26 (0.21)	1.96 (0.12)	2.13 (0.11)
2. Recife (public market) - PE	19.41 (0.88)	14.92 (0.88)	0.90 (0.05)	0.72 (0.15)
3. Palmares - PE	20.06 (0.53)	15.42 (0.53)	0.93 (0.10)	0.84 (0.14)
4. Fortaleza - CE	13.99 (0.32)	10.75 (0.32)	0.57 (0.03)	0.54 (0.12)
5. Belo Jardim - PE	20.87 (0.65)	16.05 (0.65)	1.78 (0.01)	1.71 (0.15)
6. Arcoverde - PE	37.85 (0.23)	29.09 (0.23)	2.68 (0.03)	2.57 (0.16)
7. Manaus (region 1) - AM	27.95 (0.47)	21.49 (0.47)	1.26 (0.09)	3.23 (0.13)
8. Manaus (region 2) - AM	20.74 (0.51)	15.94 (0.51)	0.97 (0.04)	1.00 (0.11)
9. Caruaru - PE	27.33 (0.00)	21.01 (0.00)	1.31 (0.01)	1.09 (0.12)
10. Maceió - AL	22.06 (0.28)	16.96 (0.28)	1.22 (0.02)	0.97 (0.17)
11. Mossoró - RN	26.48 (0.40)	20.36 (0.40)	0.71 (0.02)	0.56 (0.12)
12. Mato Grosso do Sul - MS	18.84 (2.14)	14.49 (2.14)	1.35 (0.01)	1.21 (0.13)
13. Limoeiro (region 2) - PE	21.64 (0.16)	16.63 (0.16)	1.68 (0.04)	1.23 (0.18)
14. Brasília - GO	21.45 (0.52)	16.49 (0.52)	1.24 (0.02)	0.66 (0.10)

The values were plotted with mean (RSD). TPC: Total phenolic content; HPLC: High-performance liquid chromatography; UV/Vis: Ultraviolet/visible; RSD: Relative standard deviation; EA: Ellagic acid; GA: Gallic acid; PE: Pernambuco; CE: Ceará; AM: Amazonas; AL: Alagoas; RN: Rio Grande do Norte; MS: Mato Grosso do Sul; GO: Goiás.

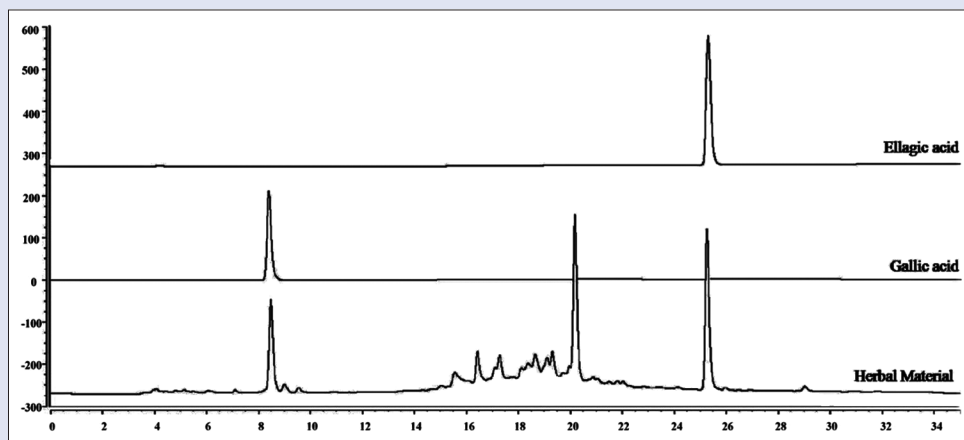


Figure 3: Chromatogram obtained for herbal material and standards (ellagic acid and gallic acid)

Table 2: Calibration data for the standards ellagic acid, gallic acid, and herbal material from *Libidibia ferrea*

	EA	GA	Herbal material	
			EA	GA
Concentration range ( $\mu\text{g/mL}$ )	23.2-34.9	24.0-36.0	1200-1800	
LOD ( $\mu\text{g/mL}$ )	-	-	1.26	1.64
LOQ ( $\mu\text{g/mL}$ )	-	-	3.83	4.97
Angular coefficient (a)	4.559	1.612	0.091	0.027
Linear coefficient (b)	7.882	-6.563	1.753	-0.966
$R^2$	0.999	0.999	0.999	0.997

LOD: Limit of detection; LOQ: Limit of quantification; EA: Ellagic acid; GA: Gallic acid

Table 3: Repeatability and Intermediate precision tests: amounts of EA (g%) and GA (g%) for the herbal material from *Libidibia ferrea*

Standard	Parameters	Content (g%) (mean $\pm$ SD; RSD%)	
Ellagic acid	Repeatability	2.110 $\pm$ 0.008 (0.40)	
	Intermediate precision	Day 1	Day 2
	Analyst 1	2.120 $\pm$ 0.011 (0.51)	2.110 $\pm$ 0.018 (0.84)
	Analyst 2	2.110 $\pm$ 0.013 (0.60)	2.120 $\pm$ 0.010 (0.49)
Gallic acid	Repeatability	2.250 $\pm$ 0.008 (0.34)	
	Intermediate precision	Day 1	Day 2
	Analyst 1	2.250 $\pm$ 0.008 (0.37)	2.210 $\pm$ 0.007 (0.30)
	Analyst 2	2.210 $\pm$ 0.008 (0.36)	2.210 $\pm$ 0.005 (0.22)

Table 4: Robustness test: Amounts of ellagic acid (g%) and gallic acid (g%) for the herbal drug from *Libidibia ferrea*

Source of variation	Parameters	g% (mean; RSD%)	
		EA	GA
Flow rate (mL/min)	0.790	2.100 (0.82)	2.160 (0.44)
	0.810	2.100 (1.14)	2.190 (0.15)
pH of mobile phase	A 2.0-B 3.0	2.120 (0.84)	2.150 (0.04)
	A 3.0-B 4.0	2.120 (0.99)	2.180 (0.55)
Column temperature ( $^{\circ}\text{C}$ )	23	2.110 (0.84)	2.170 (0.13)
	25	2.110 (0.20)	2.160 (0.11)

EA: Ellagic acid; GA: Gallic acid; RSD: Relative standard deviation

In general, few markers or pharmacologically active components are used to assess the quality and authenticity of a species that are exhibiting therapeutic potential. However, the quantification of one or

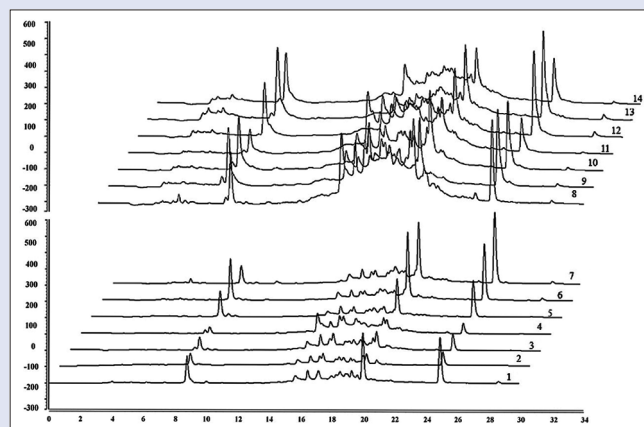


Figure 4: High-performance liquid chromatography fingerprint of 14 samples of fruit from *Libidibia ferrea*

some compounds is not representative enough to evaluate of the typical synergic effects and/or the inherent variability of biological matrices such as herbal drugs. In this context, the use of chemical "fingerprints" plays an important role on standardization of herbal drugs and herbal drug derivatives.<sup>[38-40]</sup> The multiple data analysis combine several qualitative and quantitative parameters such as number of peaks, peak areas, and/or relationship between peak performance; which improve the chemical reproducibility of the products ensuring the maintenance of safety and efficacy. Although the TLC/HPTLC provides important and visible information on the analysis of herbal materials, the use of HPLC to creating herbal fingerprints is the most popular one due to its higher precision and resolution. In addition, the LC procedures are accurate for qualitative and quantitative analysis, improving the quality evaluation of herbal drugs by the multi-component quantification.<sup>[37,41-43]</sup>

## CONCLUSION

Reproductive phenolic profiles of fruits from *L. ferrea* by HPTLC and HPLC were reported for the first time. The analytical procedures were also able to detect the variability in the profiles of several samples of the drug material. In addition, the quantitative analysis of phenolic compounds were performed by UV/VIS or HPLC and allowed to quantify the phenolic compounds (total phenol content, GA, and EA). Therefore, the results of this study shows that the qualitative (HPTLC

and HPLC) and quantitative (UV/VIS and HPLC) procedures can be used to construct references fingerprints to standardized extractives from the fruits of *L. ferrea* based on their biological properties.

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## Conflicts of interest

There are no conflicts of interest.

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