Libidibia ferrea extract as a promising antidiabetic agent: characterization, pre-clinical analysis and development of pharmaceutical dosage forms

Extrato de Libidibia ferrea como um promissor agente antidiabético: caracterização, análise pré-clínica e desenvolvimento de formas de dosagem farmacêutica

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ABSTRACT
The present work aimed at developing pharmaceutical dosage forms based on the dry bark extract of *Libidibia ferrea* for the treatment of diabetes *mellitus*. The physicochemical characterization of the plant raw material, extractive solution and dry extract obtained by freeze drying were carried out. Effervescent granules, oral solution and tablets from the dry extract were developed and subjected to the recommended quality control tests. All materials presented standardized values after physical-chemical characterization. The presence of several secondary compounds was confirmed, and the content of the tannins was determined. The extract did not present acute toxicity in the *in vivo* experiment and provided an increase in glucose uptake in the *in vitro* study. The developed formulations complied with previously established values for quality control. The results show the safety and efficacy of the developed products, may being promising options in the treatment of *diabetes mellitus*.

Keywords: Medicinal plants, Phytotherapy, Diabetes *mellitus*, Toxicity

RESUMO
O presente trabalho visava desenvolver formas de dosagem farmacêutica baseadas no extracto seco de casca de Libidibia ferrea para o tratamento da diabetes mellitus. Foi realizada a caracterização físico-química da matéria-prima vegetal, solução extractiva e extracto seco obtido por liofilização. Grânulos efervescentes, solução oral e comprimidos do extracto seco foram desenvolvidos e submetidos aos testes de controlo de qualidade recomendados. Todos os materiais apresentaram valores padronizados após a caracterização físico-química. A presença de vários compostos secundários foi confirmada, e o conteúdo dos taninos foi determinado. O extracto não apresentou toxicidade aguda na experiência in vivo e proporcionou um aumento na absorção de

glucose no estudo in vitro. As formulações desenvolvidas respeitaram os valores previamente estabelecidos para o controlo de qualidade. Os resultados mostram a segurança e eficácia dos produtos desenvolvidos, podendo ser opções promissoras no tratamento da diabetes mellitus.

Palavras-chave: Plantas medicinais, Fitoterapia, Diabetes mellitus, Toxicidade

1 INTRODUCTION

Diabetes *mellitus* (DM) is chronic metabolic disease characterized by disorders in insulin secretion, a hormone responsible for carbohydrate metabolism in the blood, altering the body's homeostasis and may leading to kidney, brain, and heart failure, as well as other organ complications (Association, 2018; Blair, 2016; Ferreira et al., 2011). DM therapy is focused on the control and maintenance of blood glucose levels as close to normal as safely possible, however, the long-term trends in antidiabetes drug usage are related with several side effects, which may lead to the worsening of diabetic-related comorbidities (Zeitler et al., 2014; Maeyama et al., 2020). In this sense, there is a great interest in the search for new alternatives for DM treatment (Castro et al., 2020).

Libidibia ferrea, (Mart. ex Tul.) L.P.Queiroz, is a well-known natural plant used as antidiabetic agents, popularly known as "pau-ferro" or "jucá" in Brazil, and having as its basionym the species *Caesalpinia ferrea* Mart. ex Tul (The Plant List, 2020). Previous ethnopharmacological studies show that *Libidibia ferrea* has several popular uses, being mainly consumed in the form of tea (decoction and infusion), elixir, syrup and linctuses (Balbach, 1992; Barros, 1982; Lewis, 1987; Oliveira et al, 2010; Venancio et al, 2020). Currently, its use based on popular knowledge can still be observed to treat kidney disease, inflammation in the urethra, diabetes, diarrhea, cleaning and treatment of wounds and bruises due to numbness, antimicrobial activity, breathing problems, anemia, enterocolitis, fever, and to support weight loss (Roque et al., 2010; Bariani et al., 2012; Paiva et al., 2015; Kobayashi et al., 2015; Farias et al., 2020; Melo et al, 2020). For the treatment of Diabetes, the preparation of teas obtained by infusing the bark of the trunk is quite common (Oliveira et al., 2010; Lima et al, 2020b).

In order to investigate the therapeutic activities seen from popular use, a number of studies have been carried out to elucidate these pharmacological properties of *L. ferrea*. In this sense, the anti-hyperglycemic action has garnering attention, which ratifies its traditional use for the treatment of diabetes, as observed in several studies that

investigated the use of fruits, peels, leaves and seeds in decreasing diabetes complications (Ueda et al., 2001; Lima et al, 2020a).

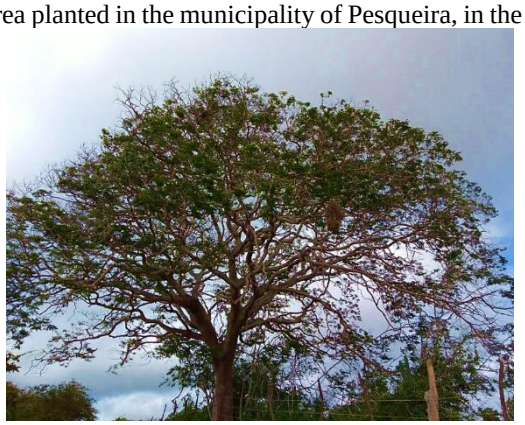
In this context, the aim of this study, therefore, was to develop pharmaceutical forms using *L. ferrea* (oral solution, effervescent granules, and tablets) as new therapeutic alternatives for the treatment of *diabetes mellitus*.

2MATERIALS AND METHODS

2.1 MATERIALS

The stem bark of *L. ferrea*, was collected in the municipality of Pesqueira, Pernambuco, Brazil, whose latitude and longitude are 8°21'28.0"S, 36°41'47.0"W, respectively. Plant exsiccata was previously identified at the Agronomic Institute of Pernambuco (IPA), under registration number #86678 (Fig. 1).

Fig. 1 – Tree of Libidibia ferrea planted in the municipality of Pesqueira, in the state of Pernambuco, Brazil.



After drying at room temperature, the material was dried in a circulating air oven at 45 ± 2 °C for weight stabilization. Then, the barks were ground in a rotary knife mill, with intermediate mesh opening. The characterization of *L. ferrea* bark powder was performed by determining the particle size distribution, loss on drying, and total ash content according to the Brazilian Pharmacopoeia 6th edition (Brasil, 2019). The Thermal analysis of *L. ferrea* raw material was performed using DTG/TG techniques, using the Shimadzu® DTG-60 equipment. The analysis was performed at a temperature range of 25 to 600 ° C under dynamic atmosphere with ultra-pure nitrogen (100 mL/min) and heating ratio of 10 °C/min using open alumina crucibles containing around 5 mg of sample (Lyra, 2013).

2.2 EXTRACTIVE SOLUTION, DRY EXTRACT, AND DOSING OF TANNINS

The extractive solution was obtained by the hot extraction method, in a drug:solvent ratio of 1:20 (w/v), using distilled water as the extraction solvent (Souza et al., 2009; Simões, 2017). For sample characterization, pH value, relative density and dry residue determinations were performed (Brasil, 2019). The extractive solution was filtered, the solvent removed by freeze drying using the LIOTOP® (model L101) equipment, under the following conditions: pressure of 30 mmHg, at -55° C during 96 hours of drying.

The residual moisture through loss on drying was also performed (Brasil, 2019). The phytochemical prospecting was performed according to qualitative methodology (Bladt, 1996; Galvão et al., 2018). The foam test was executed to confirm the presence of saponins (Simões, 2017). The Thermal analysis of *L. ferrea* raw material was performed using DTG/TG techniques (Lyra, 2013). The powder flow by angle of repose and the flow time were determined (Viana et al., 2006), as well as the bulk density and compaction value (Allen et al., 2011; Schüssele and Bauer-Brandl, 2003). Specific surface area was determined by the BET method (Brunauer, Emmett and Teller), and mean diameter and volume by the BJH method (Barret, Joyner and Halenda).

The tannin determination in the dry extract was based on the model described by Amorim et al., 2008. The calibration curve was prepared with a standard solution of gallic acid (1 mg/mL) and all other reagents, in concentrations of 0.5; 1.0; 1.5; 2.0; 2.5; 5.0; 7.5 and 10.0 µg/mL. The total phenol content was expressed as milligrams equivalent of gallic acid per gram of extract (mg GAE/ g).

2.3 PRE-CLINICAL STUDIES

2.3.1 Acute Toxicity (LD50).

To establish the LD50 (mean lethal dose), the single-dose acute toxicity experimental protocol was used, established in Guideline 423 (Organisation for Economic Co-operation and Development (OECD), 2002), and with the approval of the Ethics Committee on Animal Experimentation at the Federal University of Pernambuco (UFPE) under register number 0112019.

A single-dose of 2000 mg/kg was administered orally in the Swiss albino mice (*Mus musculus*) by gavage. The animals, weighing around 30 g and approximately four-week old, were obtained from the Department of Antibiotics, Center of Biosciences, UFPE. The animals were maintained under standard environmental conditions (12h

light/dark cycle) and temperature (22±2°C), in polypropylene cages. To carry out the investigation, the animals were divided into two groups of three animals. The mice were fasted for 12 hours prior to the test and received water *ad libitum*. Subsequently, the control group received only the vehicle (1 mL of distilled water / 100g of weight) and the treated group received the extract from the stem bark of *L. ferrea* diluted in distilled water.

After oral gavage, the animals were confined and placed on a flat surface and observed for 30 minutes for toxic signs and behavioral analysis, following the parameters recommended by Malone (1977). Subsequently, observations were made at regular intervals for 14 days. The evolution of body mass was also evaluated, as well as the consumption of water and feed throughout the experiment.

Statistical analyzes were performed using Graph Pad Prism 5.0® (GraphPad Software, Inc., La Jolla, CA 92037 USA). Values were expressed as mean ± standard deviation (SD), with analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison/post-hoc test to determine the significance level. P-values less than 0.05 were considered statistically significant.

2.3.2 In vitro Glucose Consumption

The influence of the dry extract of *L. ferrea* on the *in vitro* glucose consumption of adipocyte cells was analyzed. The 3T3-L1 cell line was acquired from the Cell Bank of the Federal University of Rio de Janeiro (UFRJ) and maintained in culture as described in the literature (Zebisch et al., 2012): medium modified by Dulbecco (DMEM; Gibco®) supplemented with 10% fetal bovine serum (FBS; Gibco®), 1% penicillin and streptomycin (Gibco®), under an atmosphere of 5% carbon dioxide (CO_2) at 37 ° C. The cells were maintained in culture until reaching the confluence of 80% and then plated in 96-well plates ($1x10^4$ cells per well). For the differentiation process, the cells remained three days in DMEM medium, containing 10% SFB, 0.5 mM 1-methyl-3-isobutyl-xanthine (IBMX), 1 µM dexamethasone, and 1 µg/mL insulin (Sigma Aldrich, USA). After that period, the cells were kept for another seven days in DMEM medium containing 10% FBS and 10 µg/mL of insulin. Cell differentiation was confirmed using the Oil Red O dye, which reveals red lipid deposits.

2.3.3 In vitro Insulin Resistance Model and Experimental Groups

The adipocytes were washed with saline buffer (PBS) and incubated with serum-free DMEM containing 0.5% bovine serum albumin (BSA) 12 h before the experiments.

The cells were divided into 4 groups: (1) positive control group containing insulin (2) negative control, and (3) and (4) the groups treated with 25 µg/mL and 50 µg/mL of the *L. ferrea* dry extract, respectively. Insulin resistance was induced by exposure of differentiated adipocytes to 1 µM of dexamethasone and 10 µg/mL of insulin for 96 hours (Zhang et al., 2013). The culture medium was collected and the glucose concentrations were determined by the glucose oxidase method.

Values were expressed as mean ± standard deviation (SD), "n" denotes the sample size in each group. The Shapiro-Wilk test was used for statistical analysis, then the analysis of variance (ANOVA) was performed, followed by the Tukey post-test. P-values less than 0.05 were considered statistically significant.

2.4 DEVELOPMENT OF ORAL PHARMACEUTICAL DOSAGE FORMS

L. ferrea dry extract and excipients were thermally characterized using the DTG/TG technique. Physical mixtures were made in a 1:1 (w/w) ratio of the dry extract with the respective excipients. The mixtures were homogenized and analyzed using the Shimadzu® DTG-60 equipment.

Table I shows the excipients used for each dosage form. After obtaining the formulations the respective quality controls were applied, following the recommendations of the Brazilian Pharmacopoeia 6th edition, as well as the Guide for Registration of Phytotherapeutic Medicines and The Registration and Notification Guideline for Traditional Phytotherapeutic Products (Brasil, 2014).

Table I - Excipients used to obtain the oral solution, effervescent granules and tablets based on the dry extract of Libibidia ferrea.

Pharmaceutical dosage form	**Excipients**
Oral solution	Sorbitol (70%), benzoic acid, sucralose and chocolate essence.
Effervescent granules	citric acid, tartaric acid and sodium bicarbonate, sucralose and polyvinylpyrrolidone.
Tablets	Microcrystalline cellulose, sodium starch glycolate, colloidal silicon dioxide and magnesium stearate.

3 RESULTS AND DISCUSSION

3.1. RAW MATERIAL AND AQUEOUS SOLUTION CHARACTERIZATION

Table II shows the physicochemical characterizations of the plant raw material and aqueous solution. The results were presented within the pharmacopoeic criteria established for *L. ferrea*.

Table II - Physicochemical characterizations of the plant raw material and aqueous solution. The results were presented within the pharmacopoeic criteria established for *L. ferrea*.

Raw material		Aqueous solution	
Granulometry	Coarse powder	Relative density	1.002 g / mL
Loss on drying	8.15 ± 0.156%	pH	5.56 ± 0.03
Total ash	8.0 ± 0.022%	Dry residue content	1.17 ± 0.03%

Following the pharmacopoeial criteria, the particle size determination test classified the powder of the *L. ferrea* raw material as coarse powder. The average value obtained from the loss of drying and total ash value are within the maximum recommended value of 13% and 8% for *L. ferrea*, respectively.

3.2. DRY EXTRACT CHARACTERIZATION

The result obtained from the residual moisture determination of the dry extract was 6.62 ± 0.56%. For the phytochemical prospecting, the results show that all metabolites contained in the plant raw material are also present in the dry extract, thus indicating that there was no negative influence from the drying process. The presence of reducing sugars, flavonoids, triterpenes, steroids, hydrolyzable tannins, and condensed tannins, and the absence of alkaloids and coumarins was confirmed by thin layer chromatography (TLC) analyses. The presence of saponins was confirmed by the foam test (Simões, 2017). According to the literature, condensed (catechins) and hydrolyzable (gallic acid and ellagic acid) tannins are the main compounds of the dry extract of *L. ferrea* stem bark (Hassan et al., 2015) and responsible for its antidiabetic activity (Ueda et al., 2001; Hassan et al., 2015; Carvalho, 1993). In the tannin assay, a total of 370.14 mg of Gallic Acid equivalent per gram (EAG/g) of the dry extract of *L. ferrea* were found, showing high tannin content (Neri, 2016).

The nitrogen adsorption test shows that according to the International Union of Pure and Applied Chemistry (IUPAC), the pores in the dry extract of *L. ferrea* can be classified as mesoporous, with a pore diameter of 142.8590 Å, ranging from 2-50 nm (20 Å and 500 Å) (Nery et al., 2008). In this sense, determinations of surface area and porosity are critical for pharmaceutical preformulation studies in formulation and development of new dosage forms. Therefore, the extract has a high porosity, with 2.3399 m^2/g of surface area and 0.0064 cm^3/g of pore volume, which confers good solubility by facilitating liquids penetration (Porte et al., 2011; Santos, 2013).

The angle of repose and the flow time of the dry extract of *L. ferrea* were not possible to be obtained, since the extract showed high hygroscopicity and lack of fluidity.

Unsatisfactory determinations of these tests are expected for dried plant extracts (Chaves et al., 2009). Regarding the Hausner factor and densification index, the results presented for these parameters indicate that the powder has more stable packing properties and this characteristic impairs the flowability (Table III). Thus, diluents and lubricants were needed to increase flow properties.

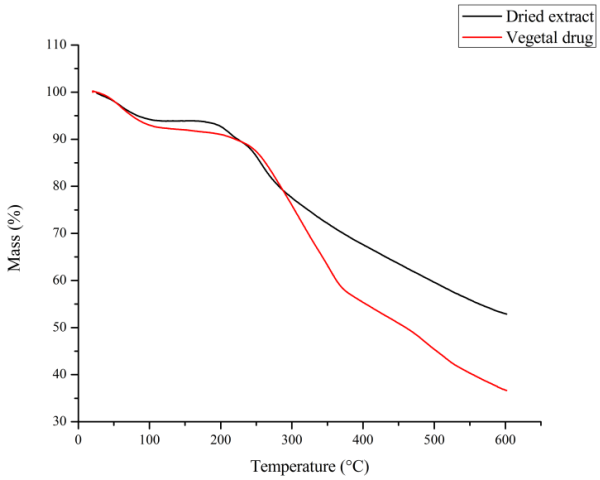
Table III - Analysis of the rheological behavior of Libidibia ferrea dry extract.

Properties	**Especifications**	**Results**
Bulk density	-	0.23 g/mL
Compacted density	-	0.45 g/mL
Hausner factor	< 1.25	1.95
Carr Index	5-15%	48.79 %
Densification index	< 20mL	21 mL
Rest angle	< 30º	There was no outflow
Flow time	< 10 s	Infinite

The angle of repose is indicative of the packing properties. By aggregating this test to the flow time, it is possible to analyze the flow of powders, which represents a fundamental factor in solid pharmaceutical formulations (Chaves et al., 2009). The values of apparent and compacted densities, Housner ratio and Carr index can predict the propensity of a given powder sample to be compressed. The density determination evaluates the dynamic behavior of particles (Fahr and Voigt, 2015). Thus, these parameters are indicative of packing stability of powders and their rheological characteristics.

The thermogravimetry (TG) curves of *L. ferrea* vegetal drug and its dried extract can be seen in Fig. 2. The first thermal event, in 22-80 ºC (Δm = 6.5%), represents the loss of free water present in the vegetal drug and the thermal decomposition begins at 260 ºC with a total mass loss of 22% until 600 ºC. Regarding the thermal analysis of the freeze-dried extract, a similar thermal event was initially found, where the loss of free water was observed up to 100 ºC (Δm = 3.2%), thus showing remnant moisture after the drying process. The thermal decomposition presented two events, the first between 121 and 268 ºC and the second in 288 and 408 °C, with the percentage mass loss of 10.72% and 25.87%, respectively.

Fig. 2 - Thermogravimetry (TG) curve of the vegetable drug and dry extract.



The first thermal event of the dry extract was found in lower temperatures when compared to the vegetal drug, which may be related to the smaller amount of free water in the dry extract. The results from the thermal analysis of the vegetal drug and *L. ferrea* dry extract are fundamental for temperature control during the process of production of solid pharmaceutical forms and storage of the plant material.

3.3. PRE-CLINICAL STUDIES

3.3.1. Acute toxicity (LD_{50})

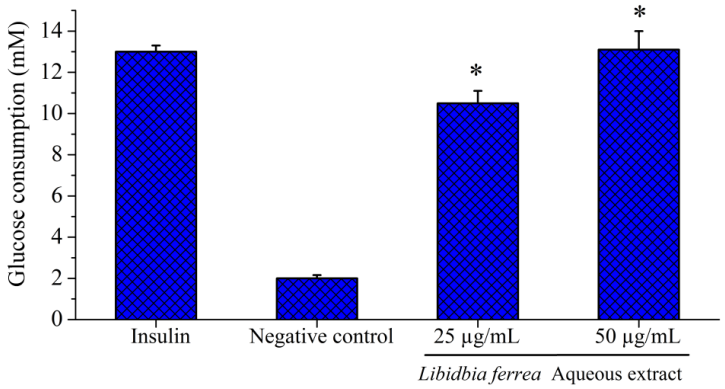
Regarding the acute toxicity test in mice, administrated at 2000 mg/kg, no clinical signs of toxicity, weight changes or alterations in water and feed consumption during the observation period was identified. Acute toxicity investigation is a widely-used methodology for identifying and classifying substances for their potential to cause acute damage to living organisms, in high doses, and may offer assistance in establishing safety parameters along with other toxicity data for human health (Cruz et al., 2017; Nascimento et al., 2016).

Given the observed results and according to Guideline 423 (Organisation for Economic Co-operation and Development (OECD), 2002), the concentration used is considered safe (category 5) with estimated LD_{50} higher than 2000 mg/kg. The aqueous extract of *L. ferrea* was considered orally non-toxic in mice in a single acute dose, thus corroborating with the popular use of this type of extract, as well as with several studies as reported (Hassan et al., 2015; Freitas et al., 2012; Cavalheiro et al., 2009; Lima et al., 2011; Pereira et al., 2012).

3.3.2. In vitro Glucose Consumption

The effect of *L. ferrea* dry extract on glucose consumption in 3T3-L1 adipocytes cell line was determined by the glucose oxidase method (Fig. 3). In the negative control group, the amount of glucose consumption decreased significantly with dexamethasone and increased with insulin, demonstrating that the cellular model of insulin resistance was successfully developed.

Fig. 3 - Effect of *L. ferrea* dry extract (25 and 50 µg/mL) on glucose consumption in 3T3-L1 adipocytes cell line. Values are expressed as mean ± s.d. One-way analysis of variance (ANOVA) followed by Tukey.



In the analyzed concentrations *L. ferrea* dry extract increased fat cells glucose consumption, thus corroborating the antidiabetic effect observed in the literature (Ueda et al., 2001; Souza et al., 2009; Hassan et al., 2015; Vasconcelos et al., 2011; Cunha et al., 2017).

3.4. DEVELOPMENT OF ORAL DOSAGE FORMS CONTAINING DRY EXTRACT OF LIBIDIBIA FERREA

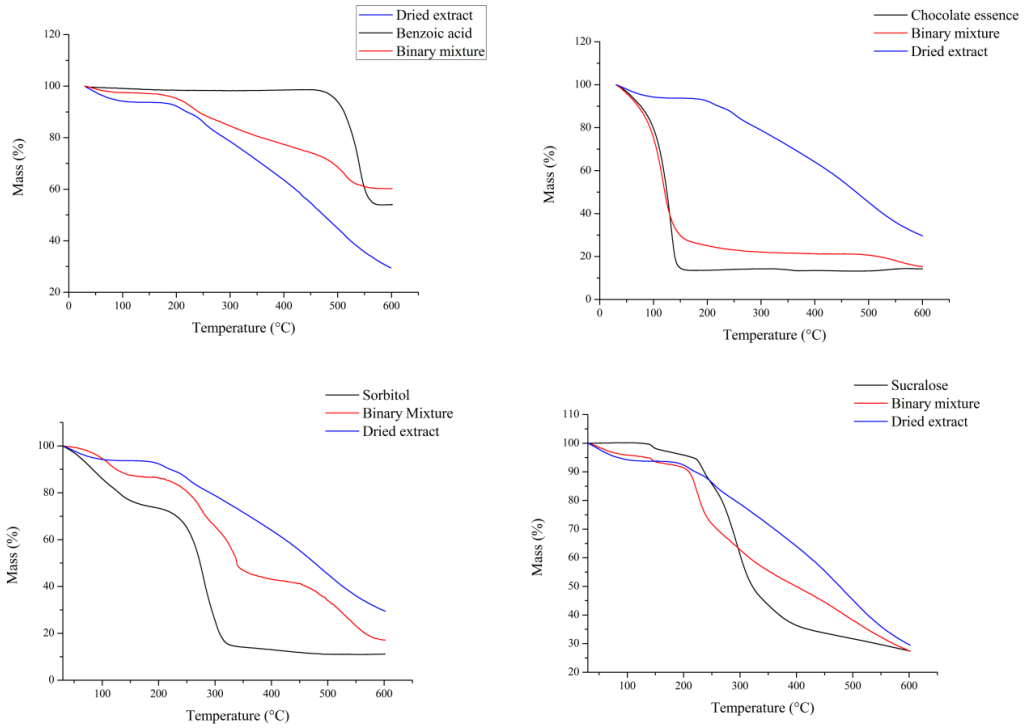
The appropriate dosage form can be selected based on several factors such as ease of administration, convenience and safety (Hasenclever et al., 2017). Taking into consideration the individualities of the group of people affected by diabetes *mellitus* syndrome, this study sought to diversify the options of treatment, and thus, the following pharmaceutical forms were developed: oral solution, effervescent granules and tablets.

3.5. TECHNOLOGICAL DEVELOPMENT OF THE ORAL SOLUTION CONTAINING DRY EXTRACT OF LIBIDIBIA FERREA

Figure 6 shows the thermogravimetry (TG) curves of the compatibility study between *L. ferrea* dry extract and the excipients selected for oral solution formulation. A

probable interaction between the dry extract and the chocolate essence was observed, as can be seen in Fig. 4.

Fig. 4 - Thermogravimetric analysis curve (TG) of the dry extract of *Libidibia ferrea*, excipients and binary mixtures for the oral solution.



The thermal analysis curve of the binary mixture of *L. ferrea* dry extract with chocolate essence was similar to that of the isolated excipient, thus indicating that the dry extract was solubilized by the essence preventing the analysis of the extract-excipient interaction. In this study, there was no interaction between the dry extract of *L. ferrea* and the remaining excipients selected for the oral solution, since the curves of the binary mixtures presented loss of mass at temperatures equal to or higher than the isolated dry extract. Thus, six bench batches containing 0.500 g of dry extract of *L. ferrea* were manipulated at each dose in a final dose volume of 15 mL. None of the batches showed solubility problems. The LB VI presented the best palatability. The final composition of the selected bench lot can be seen in Table IV.

Table IV - Bench lot VI of *Libidibia ferrea*-based oral solution.

Composition	**%**	**g**
Dry extract of *L. ferrea*	3.330	5,000
Sorbitol 70%	30.000	45,000
Benzoic acid	0.500	0,750
Sucralose	0.800	1,200

Chocolate Essence	0.100	0,150 mL
Purified water	q.s.	150 mL

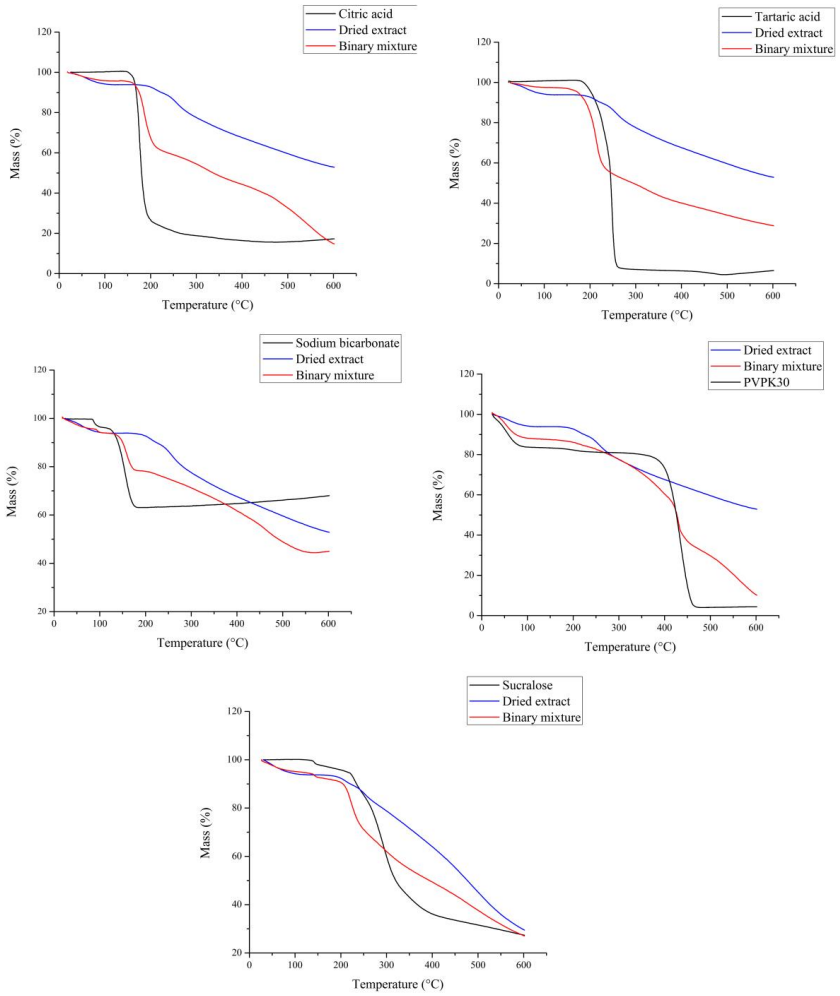
The organoleptic characteristics obtained are associated with the plant raw material and the excipients used. The pH value obtained in the oral solution did not differ from that found in the extractive solution, maintaining the same acid character (Longhini et al., 2007). The density found was slightly higher when compared to the extractive solution, which may be due by the inclusion of excipients (Allen et al., 2011).

Regarding the determination of tannin content, when comparing with the value found in the dry extract, the result obtained at the oral solution was within the 15% of variation for the active marker, which is in accordance to the Guide for Registration of Phytotherapeutic Medicines and The Registration and Notification Guideline for Traditional Phytotherapeutic Products established by ANVISA (Brasil, 2014).

3.6. TECHNOLOGICAL DEVELOPMENT OF EFFERVESCENT GRANULES CONTAINING DRY EXTRACT OF LIBIDIBIA FERREA

The results obtained from the compatibility study between the dry extract and excipients can be seen in Fig. 5, which shows that there was no interaction between the dry extract and the excipients selected, since the curves of the binary mixtures presented loss of mass at temperatures equal to or higher than the dry extract.

Fig. 5 - Thermogravimetric analysis curve (TG) of the dry extract of *Libidibia ferrea*, excipients and binary mixtures for the effervescent granules.



In this sense, the excipients chosen for the effervescent granules do not interact with the dry extract of *L. ferrea* and could thus be used for the formulation. Eight bench batches containing 1.00 g of *L. ferrea* dry extract were manipulated at each dose.

Among the bench batches obtained, LB VIII presented the best performance in effervescence, solubility, sweetening capacity and the best flowability. The total yield of the granulation process was 71.18%. The final composition can be seen in table V.

Table V - Final formulation of *Libidibia ferrea* effervescent granules. Bench lot VIII.

Composition	**%**	**g**
L. ferrea extract	35.59	10.00
Sucralose	28.47	8.00
Citric acid	5.34	1.50
Tartaric acid	10.67	3.00
Sodium bicarbonate	18.15	5.10
PVP K30	1.78	0.50
Total	100.00	28.10

For physicochemical characterization, the effervescent granulate was solubilized in water, presenting the macroscopic aspect shown in figure 9B. For the organoleptic characteristics, no color or taste changes were found when comparing aqueous extract and freeze-dried powder. In addition, after granules dissolution in water, the preparation presented the same acid character as in the extractive solution, which together with the presence of tannins resulted in an astringent flavor. The density value was close to that of the extractive solution, as well as that of water. In the residual moisture verification, a significant decrease was observed when compared to the value obtained in the dry extract, presenting a value of 2.39 ± 0.48%, within the recommended (0.5-2.0), which is suggestive that after the granulation process, the final formulation can present a reduced risk of microbial contamination.

In the granule disintegration and effervescence test, after addition of 200 mL of distilled water, the gas bubbles were completely released and when the gaseous release ceased all solid components were disaggregated and dissolved or dispersed within less than 5 minutes, as recommended by the European Pharmacopoeia (*European Pharmacopoeia (Ph. Eur.)*, 2017). Regarding the determination of tannin content, as well as the oral solution, the result obtained was within the 15% variation parameter (Brasil, 2014).

After the formulation process all the deficient flowability of the dry extract were overcome (table VI). The effervescent granules showed higher values of apparent and compacted densities, and presented excellent flow and ease of compaction. These results indicate that the final formulation has a greater potential to present satisfactory rheological properties with remarkable flowability.

Table VI - Analysis of the rheological behavior of Libidibia ferrea effervescent granules.

Properties	**Especification**	**Results**
Bulk density	-	0.69 g/mL
Compacted density	-	0.81 g/mL
Hausner factor	< 1.25	1.17
Carr Index	< 20%	14.81 %
Densification index	< 20mL	1 mL
Rest angle	< 30º	21.23°
Flow time	< 10 s	1.07s

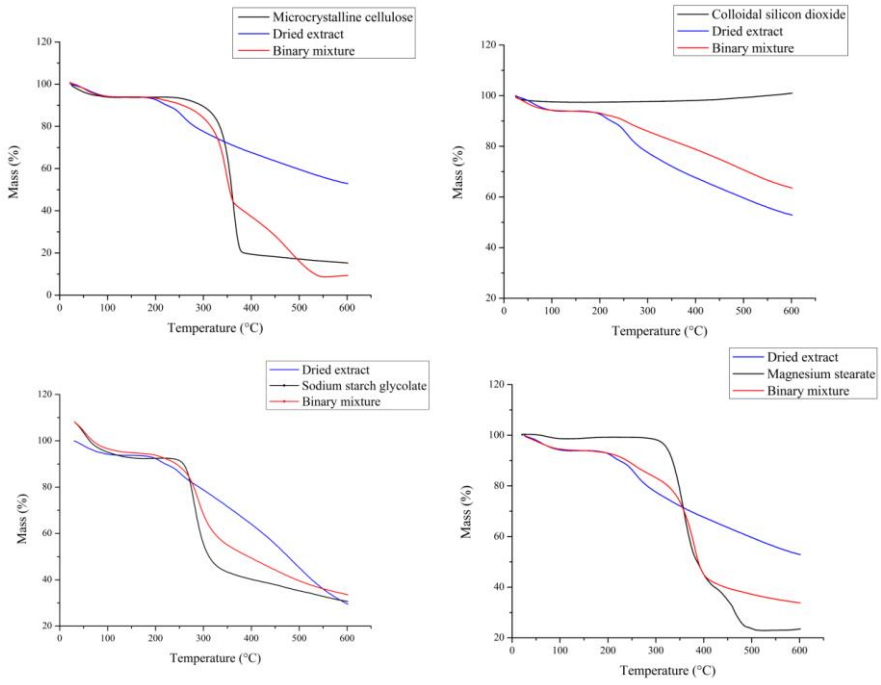
Given the pore diameter observed, the granulate can also be classified as mesopore, with 1.4769 m^2/g of surface area. However, different to what was found in the dry extract, the low surface area of *L. ferrea* granulate together with its higher volume

(0,012140 cm³/g) and medium pore diameter (492,142 Å) gives better wettability and thus a high solubility.

3.7 TECHNOLOGICAL DEVELOPMENT OF TABLETS CONTAINING LIBIDIBIA FERREA DRY EXTRACT

As can be seen in Fig.6, there was no interaction between the dry extract and the excipients selected, since the thermogravimetry (TG) curves of the binary mixtures did not show mass loss events when compared to the dry extract, indicating that the excipients did not anticipate the thermal events of the dry extract.

Fig. 6 - Thermogravimetric analysis curve (TG) of the dry extract of Libidibia ferrea, excipients and binary mixtures for tablet formulation.



Thus, as with the other pharmaceutical forms, the compatibility study shows that the excipients selected for the formulation of tablet containing *L. ferrea* dry extract do not interact with the dry extract and can thus be used in the formulation. Three bench batches of *L. ferrea* tablets presented an average weight of 0.500 g. Each tablet contains 0.334 g of dry extract (Table VII).

Table VII - Final formulation of *Libidibia ferrea* tablets. Bench lot II.

Composition	**%**	**g**
L. ferrea extract	66.80	3.340
Microcrystalline cellulose	27.00	1.350
Sodium Starch Glycolate	3.00	0.150

Colloidal silicon dioxide	0.40	0.020
Magnesium stearate	2.80	0.140
Total	100	5.000

Following the pharmacopoeial criteria the acceptable range for tablets with an average weight of 250 mg or more is ± 5.0%, where no more than two units can be tolerated outside the specified limits (Brasil, 2019). *L. ferrea* tablets had an average weight of 0.499 g, and thus, withing the established limits. Regarding the friability test, after 100-time rotation, no tablet was broken, chipped or cracked, and presented a weight loss value below 1.5% (0,11%), characterizing them as resistant. The hardness value (kgf/cm^2) obtained was greater than 10 kgf/cm^2 (14,22 ± 0,17 kgf/cm^2) and did not negatively influence tablet disintegration time, which began around 5 minutes and by 16 minutes the tablets were soluble in the medium.

Regarding the determination of tannin content, the value was within the 15% of the defined variation parameter (Brasil, 2014) for the active marker. In the tablet dissolution test, after the 60-minute period, the medium presented 38.30% of tannins dissolved.

4 CONCLUSION

In our study, pharmacognostic specifications were obtained within the established for quality criteria, as well as for safety and pharmacotechnical parameters. Moreover, its effectiveness through the *in vitro* glucose consumption test showed antidiabetic effect on adipocyte cells.

From the standardization of the dry extract, the preliminary development of three dosage forms was carried out. The preformulation studies showed satisfactory results. The findings in the preliminary development indicated that oral pharmaceutical forms (oral solution, effervescent granules, and tablets) containing *Libidibia ferrea* extract may be promising alternatives in the treatment of diabetes *mellitus*.

DECLARATION OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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