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TECHNOLOGICAL DEVELOPMENT OF OINTMENT BASED ON ETHANOLIC EXTRACT OF *LIBIDIBIA FERREA* FOR ANTIMICROBIAL ACTIVITY

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Abstract:

The development of new antimicrobial agents is important for public health. Libidibia ferrea (jucá or pau-ferro) is a species of Fabacea family widely used in folk medicine for its numerous therapeutic properties, which indicates the presence of compounds of pharmacological interest, including flavonoids and tannins, which are responsible for several biological activities, as well as antimicrobial activity. Thus, the objective of this work was to technologically develop an ointment based on L. ferrea and to evaluate its antimicrobial potential. The physicochemical characterization of the powder (particle size distribution, thermogravimetric analysis (TGA), loss on drying and total ash determination), the characterization of the fluid extract (determination of pH, relative density and dry residue, phytochemical prospection and determination of total flavonoid content (TFC)), and tests for antimicrobial activity of the extract and ointment based on the extract were performed. The powder was classified as moderately thick, with values of 0.3499% and 0.0635% of loss on drying and total ash determination, respectively; thermogravimetric analysis revealed three mass loss events; the fluid extract present pH value of 4.29; relative density of 0.98276 g/mL and dry residue of 3.0984%. Tannins, saponins and flavonoids were found in the phytochemical prospection. The ethanolic extract of Libidibia ferrea seeds and the ointment based on the extract showed promising antimicrobial activities against Staphylococcus aureus, followed by Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae strains. In conclusion, the developed ointment presented the potential to be used as an antimicrobial agent against microorganisms responsible for major infections.

Keywords: *Libidibia ferrea. Herbal medicines. Seed. Semi-solid pharmaceutical forms. Antimicrobial.*

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INTRODUCTION:

Brazil is one of the countries with the greatest biodiversity in terms of plant species, which may present a potential source of novel therapeutic agents [1]. It is well known that plant-derived compounds are potential sources of new antibiotics, among other pharmacological classes [2, 3].

Traditional medicine has remained the most accessible and easily accessed source of treatment in the primary health care system [4]. Plants have long been used as the primary therapeutic source, and furthermore, in several cases the therapeutic intervention depends on plants or plant-derived products [5]. Research in the field of medicinal plants is essential, since there is a constant need for new bioactive molecules [6]. Moreover, since microbial resistance to the conventional antibiotics used in the therapy is becoming more common, the discovery of new alternatives is a promising and urgent research area [7].

Libidibia ferrea (Mart. Ex Tul.) L.P. Queiroz, formerly called *Caesalpinia ferrea* Mart. ex Tul. is a leguminous plant widely distributed in the Northern and Northeastern regions of Brazil, where it is also known as "jucá" or "pau-ferro". It is a species widely used in folk medicine for its numerous therapeutic properties such as anti-inflammatory, antiulcerogenic, analgesic, antimicrobial, healing, hypoglycemic, anticoagulant and antipyretic, which indicates the presence of compounds of pharmacological interest [8–12]. Due to its importance in ethnomedicine, *Libidibia ferrea* is part of the National List of Medicinal Plants of Interest to Health System (RENISUS) in Brazil, a list released in 2009 by the Ministry of Health from the National Policy of Medicinal and Phytotherapeutic Plants. In this list, there are 71 vegetable species that have the potential to become herbal medicines [13].

These biological activities motivated the phytochemical studies of *Libidibia ferrea*, which showed the presence of bioactive compounds, such as glycosides, condensed tannins, phenolic compounds, and flavonoids [1].

In this context, our group concentrated efforts on studies of standardization of plant material and ethanolic extract of *Libidibia ferrea* seeds, as well as on the development of analytical methods and future semi-solid pharmaceutical forms containing the ethanolic extract of *Libidibia ferrea* seeds to ensure effectiveness, accessibility and better adherence to treatment, resulting in an innovative therapeutic proposal in the antimicrobial.

The objectives of this work were to perform the physicochemical characterization of the seeds and the ethanolic extract of the seeds of *Libidibia ferrea*, to carry out the technological development of the extract-based ointment and to investigate antimicrobial activity of the extract and final formulation.

MATERIAL AND METHODS:

Plant Raw Material:

The samples of the *L. ferrea* seeds were collected in the city of Caruaru, Pernambuco, whose geographical coordinates are Latitude: 08º17'01 "S and Longitude: 35.58'34 "W.

The exsiccate is deposited in the Herbarium Dárdano de Andrade Lima, Instituto Agronômico de Pernambuco under registration number 53094.

The plant raw material (100 g) was dried in an air circulating oven at 45°C for one week until constant weight.

Physicochemical Characterization of Plant Raw Material:

The characterization of the powder of the *L. ferrea* seed was carried out through particle size analysis, loss on drying, total ash content, and thermal profile obtained by thermogravimetric analysis (thermogravimetry; TGA), according to the Brazilian Pharmacopoeia 6th Edition.

Particle Size Distribution:

According to the Brazilian Pharmacopoeia 6th Edition, particle size distribution is determined with the aid of sieves operated by a mechanical device (tamper) reproducing horizontal and vertical movements. Six sieves were separated with the following mesh numbers: 20, 28, 32, 35, 60, and 100 corresponding to the mesh apertures of 850, 600, 500, 425, 250, and 150 μm, respectively. The larger aperture set was mounted over the smaller aperture set and the set was placed over the sieve receiver of the Bertel® Screen Stirrer. 10 g of the plant raw material were weighed in an analytical balance (FA2104N-Bioprecisa Eletronic Balance®) and the sample was transferred to the top sieve, evenly distributing the powder. The assembly was capped. The Bertel® sieve agitator was operated for 15 minutes with 60 vibrations. The percentage of retention of the sample in each sieve was calculated [14].

Loss on Drying (Determination of Moisture Content):

Loss on drying was performed through gravimetric method, which promotes the loss of water by desiccation. About 2 g of sample were transferred to a pre-desiccated and weighed weighing bottle for 30 minutes. The *L. ferrea* seed powder was dried in an air circulating oven (Ethik Technology®) at 100-105°C for 5 hours, until it reached constant weight.

The weighing bottle was placed in the desiccator until reaches room temperature and the weighing operation was carried out until constant weight was obtained. The percentage of loss was calculated. The test was performed in triplicate [14].

Determination of Total Ashes:
In the determination of total ashes, 3g of the pulverized plant raw material were weighed and then transferred to previously calibrated silica crucible. The sample was uniformly distributed in the crucible and incinerated gradually increasing the temperature to a maximum of 600 ± 25°C until all of the charcoal was removed. A temperature rate (30 minutes at 200°C, 60 minutes at 400°C and 90 minutes at 600°C) was applied. It was cooled in a desiccator and weighed. The percentage of ash in relation to the air-dried material was calculated [14].

Thermogravimetry Analysis (TGA):
The thermal profile was obtained by thermogravimetric analysis (TGA) using the Shimadzu® DTG-60H thermobalance in the temperature range from 30 to 600ºC, under a dynamic atmosphere of N_2 at a flow of 50 mL/min, at a temperature heating rate of 10°C/min using platinum crucible and 11.40 mg of the sample. The selection of the kinetic model for the calculation of the activation energy (Ea), constant rate (k) and reaction order was performed by computer simulation using the OZAWA method of TA-60H. The thermobalance was previously calibrated with a sample of calcium oxalate monohydrate (Merck®) with purity of 99.999%.

Obtainment of Ethanolic Extract from *L. ferrea* seeds:
The ethanolic extract of the seeds of *L. ferrea* seed was obtained through the methodology of the Herbal Medicine Form of the Brazilian Pharmacopoeia 2nd Edition. The maceration process was done to obtain the ethanolic extract in a ratio of 1:2 (1g to 2 mL). 600g of plant material were weighed and added to 1200 mL of 70% ethanol. The plant material was macerated for 7 days.

The physicochemical characterization of the ethanolic extract was carried out through the determination of the dry residue, pH determination, density determination, phytochemical prospection, and spectrophotometric profile.

Physicochemical Characterization of the Ethanolic Extract:
The determination of the pH was done in pH meter (Micronal®, model B474). For determination of density, the methodology described in the Brazilian Pharmacopoeia 6th Edition [14] was followed.

The determination of dry residue was carried out following the methodology described in the Brazilian Pharmacopoeia 6th Edition. 2mL of ethanolic extract were transferred to Petri dish, evaporated to dryness in a water bath and dried in an air circulating oven (Ethiktchnologi®) at 100-105°C for 3 hours. It was cooled in a desiccator and weighed. The dry matter calculations were performed in percentage on the mass [14].

Phytochemical Prospection:
The ethanolic extract from the seeds of *L. ferrea* was submitted to the investigation for the presence of condensed and hydrolysable tannins, flavonoids, steroids and saponins, through thin layer chromatography (TLC). The eluents and developers used in the analyzes were proposed by Wagner and Bladt [15].

The spectrophotometric profile of the ethanolic extract from *L. ferrea* seeds was obtained following the methodology of Figueirêdo et al [16], with adaptations for the plant material of this study for the determination of total flavonoids. 0.4 mL of the ethanolic extract was diluted in 20 mL of methanol to prepare a stock solution. A 6 mL aliquot of the stock solution was poured into a 25 mL volumetric flask, 2 mL of aluminum chloride were added and the final volume was reached with methanol. The sample was made in triplicate. The blank solution was prepared with a 6 mL aliquot of the stock solution in a 25 mL volumetric flask and the final volume was reached using methanol. The absorbances were obtained using a Shimadzu® brand mini-1240 UV spectrophotometer, at the wavelength of 414 μm.

The total flavonoid content (TFC) was expressed as percentage (%w/w) of the flavonoid content, calculated with quercetin.

Obtainment of the dry extract of the seeds of *L. ferrea*:
The extract was dried using a spray dryer (LabPlan®). The following parameters were used: atomization temperature of 160ºC, 30% of Aerosil® (used as drying aid) and medium air flow of 350 mL/h.

Then, the dried extract was characterized through residual moisture determination and the yield of the drying process.

Pharmacotechnical Development of the Ointment:
Three batches, named BL I, BL II and BL III, were formulated and prepared following the methodology of the cold manufacturing process in a laminar flow chamber at the Pharmaceutical Laboratory of the State of Pernambuco (LAFEPE),

where the components of the formulation (solid petrolatum, lanolin and extract) were autoclaved, resulting in sterile formulations. For BL I and BL II, 50% of the formulation corresponded to the dry extract of *L. ferrea* seeds, and for BL III 75% of the formulation corresponded to the dry extract of *L. ferrea* seeds, with different concentrations of solid petrolatum and lanolin, to determine the most suitable formulation for the ointment.

Subsequently, BL III was selected as the best formulation due to its viscosity and spreadability. The formulations are shown in Table 1.

Table 1: Batches of formulations based on the ethanolic extract of seeds *Libidibia ferrea*

COMPONENTS	%		
	BL I	BL II	BL III
L. ferrea extract	50.00	50.00	75.00
Solid petrolatum	20.00	10.00	10.00
Lanolin	30.00	40.00	15.00
TOTAL	100.00	100.00	100.00

Determination of Antimicrobial Activity:
Microbial Strains:
The antimicrobial activity was evaluated against the following microorganisms: *Staphylococcus aureus* UFPEDA-02 (Gram-positive bacteria), *Escherichia coli* UFPEDA-224, *Klebisiella pneumoniae* UFPEDA-396, *Pseudomonas aeruginosa* UFPEDA-416 (Gram-negative bacteria and *Candida albicans* UFPEDA-1007 (yeast).

Microbial suspensions were standardized: microorganism colonies were suspended in 0.9% saline solution and, by spectrophotometric method, the suspensions were adjusted to a final concentration of $1.5x10^8$ CFU/mL for bacteria [17] and 1.5×10^5 CFU/mL for yeast [18].

Antibacterial Activity Test:
The minimum inhibitory concentration (MIC) of the formulated ointment based on the ethanolic extract of the seeds of *L. ferrea* was determined using the agar diffusion method using well plates [19]. Bacterial and yeast inoculums were uniformly spread using a sterile swab for 20 mL in Mueller Hinton solidified agar for bacteria, while Sabouraud dextrose agar was used for yeast culture. On the agar plates, the reservoir wells measuring 8 mm in diameter were formed using a sterile cork probe.

Serial dilutions ranging from 75% to 0.29% of the ointment were performed. 50 µL aliquot of each dilution was added to each well. As a positive control, gentamicin (4 µg/mL) was used for bacterial strains and amphotericin B (1 µg/mL) for yeast. Ethanol was included as a negative control. Plates were incubated at 37°C for 24 hours for bacteria and at 30°C for 24-48 hours for yeast. Each sample was tested in triplicate and the antimicrobial activity expressed as the ratio of the zone of inhibition (mm) produced by each sample.

RESULTS AND DISCUSSION:

The results obtained from the determination of the particle size distribution of the plant raw material, with the retained and pass fractions, are described in Figure 1.

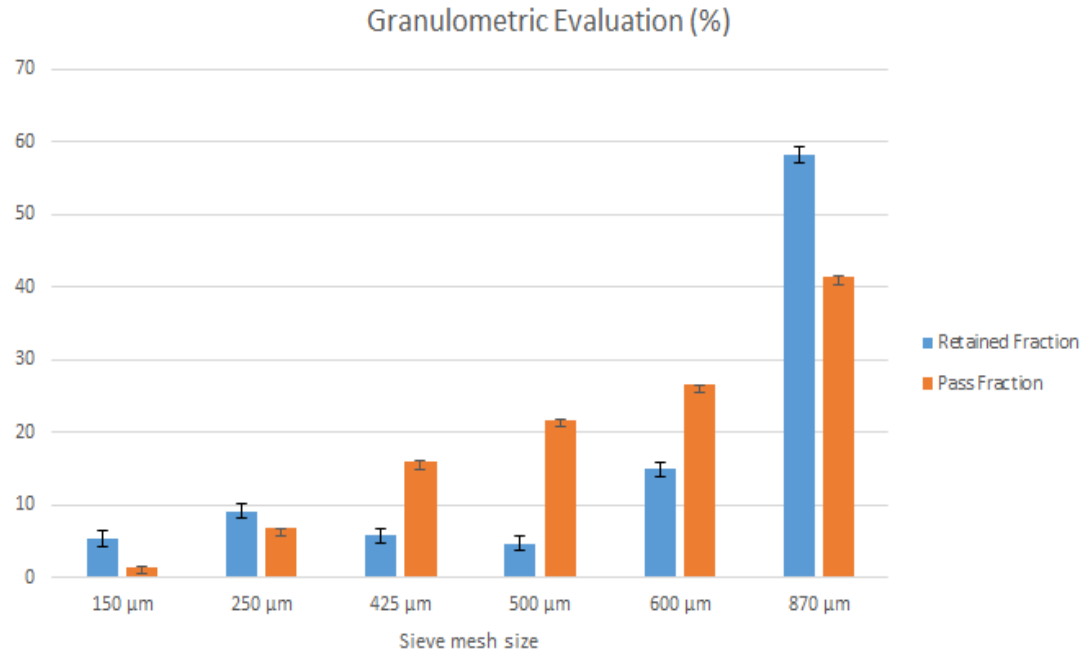


Figure 1: Determination of particle size distribution of *L. ferrea* seed powder

According to the Brazilian Pharmacopoeia 6th Edition, powders can be classified as: Thick Powder, Moderately Thick Powder, Semi-thin Powder, Thin Powder, and Very Thin Powder. According to the results obtained, *L. ferrea* seed powder was classified as thick powder, since more than 50% of the particles were retained in the highest sieve mesh size [14].

Regarding the results found in the loss on drying test the amount of volatile substance present in the sample is low, 0.3499 ± 0.0015, when compared to that shown in the pharmacopoeia, which recommends that all raw materials have an adequate residual humidity for storage in the range of 8 to 14% [14].

The loss on drying or determination of moisture content in vegetable drugs aims to determine the amount of volatile substance of any nature eliminated at 100-105ºC and is important for microbiological control, since excess water in plant material may favor the growth of microorganisms, as well as lead to the hydrolysis of its constituents [14, 20]. Since *L. ferrea* seed powder has a low moisture content, it does not present a significant risk of microbiological proliferation.

Regarding the total ash content, the value observed in the study is considered low: 0.0635 ± 0.0015. In this sense, it is within the recommended parameters, which recommends a maximum of 8% [14].

The determination of total ash includes physiological ash and non-physiological ash [14]. It is a technique that corresponds to the carbonization of the material to the ashes. It is an important indicator to evaluate the presence of non-volatile inorganic materials, such as sand or stones, which reflect problems in the collection, purchase and preparation of samples [21].

The percentage differences of these two parameters when compared to other studies may be explained by differences in water and sap distribution, and constitutions in different parts of the plant, as well as due to seasonal differences, collection place and sample preparation methods of plant material [22].

The results of the thermal analysis obtained by the thermogravimetric method of *L. ferrea* seed powder are presented by the TG/DTA curves, where they present three events that correspond to the mass losses. The temperature ranges in which events occur can be seen in Figure 2.

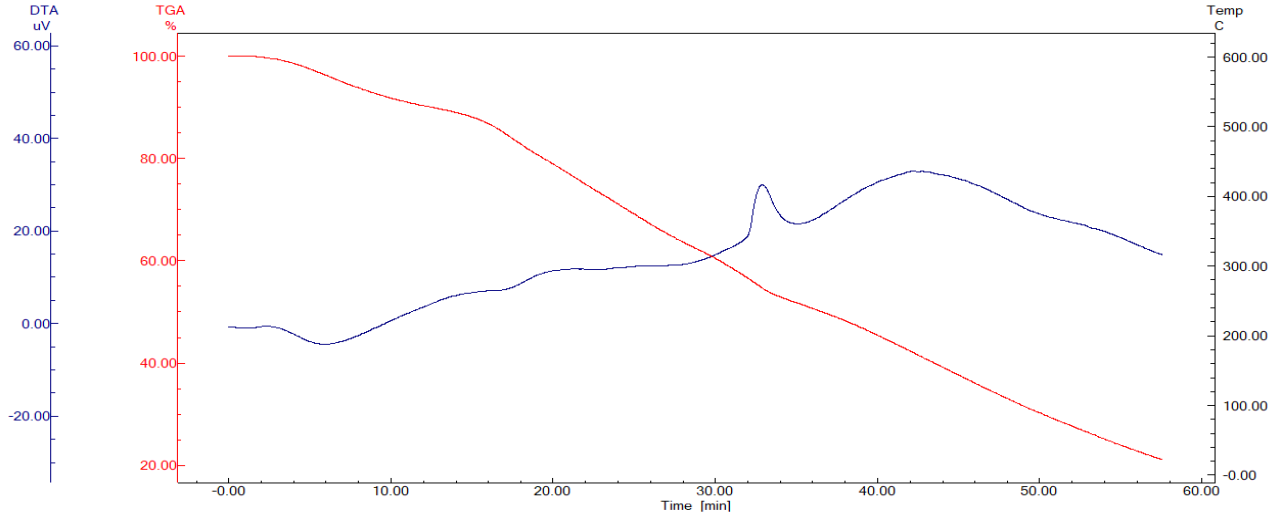


Figure 2: Mass Loss at Thermogravimetric Analysis Temperature Ranges

Subtitle: TGA- Thermogravimetric Analysis; 1- Interval from 20ºC to 240ºC; 2- Interval from 260ºC to 500ºC; 3- Interval from 500ºC to 600ºC.

Thermogravimetric analysis verified the percentage of mass loss equivalent to each event, as described in Table 2.

Table 2: Mass loss percentage obtained through thermogravimetric analysis.

Event	Temperature range (Cº)	Mass loss percentage (%)
1	20 – 240	31.58
2	260 – 500	32.89
3	500 – 600	9.53

The first event was initially related to the loss of water and volatile substances at temperatures up to approximately 100ºC, followed by the beginning of mildly represented thermal degradation; the second event was related to the first thermal decomposition step; the third event was related to the second stage of degradation of the plant raw material.

Thermogravimetric analysis (TGA), according to the Brazilian Pharmacopoeia 6th edition: "*is the thermal analysis technique in which the sample mass variation is determined as a function of temperature, or heating time, using a controlled temperature program*". TGA provides information on the thermal stability of the sample, its composition and the stability of intermediate and final product components [14]. With the application of differential thermal analysis (DTA) technique, it is possible to obtain temperature difference records as a function of time, as the technique performs continuous temperature measurement of samples compared to an inert reference material, which results are expressed as peaks for endothermic and exothermic reactions [23].

In the thermal analysis of *L. ferrea* seed powder, the DTA curve evidenced the water loss, as well as the events related to decomposition with characteristic energy absorption and release peaks, corroborating the results obtained on the TG curve.

In the preparation stage of the ethanolic extract of the seeds of *L. ferrea*, a volume of 605 mL of ethanolic extract was reached, and the yield of approximately 50% was obtained. Then, the characterization of the fluid extract was performed. The pH value obtained, 4.29, corroborates with values found in other studies of other parts of the plant. pH measurement is an important factor with regard to extract stability analysis, since at acid pH there is a decrease in the action of some microorganisms in liquid pharmaceutical forms [24]. Thus, the liquid extract has an acidic nature and a relative density close to ethanol and water, 0.9828g/mL, as expected, since they were the solvents used to prepare the extract.

The percentage of dry residue for ethanolic extract of *L. ferrea* seed is higher (3.0984% ± 0.158) than that found in other studies about other parts of the plant, which implies that ethanolic extracts of this plant species have the capacity to extract more substances. Therefore, the drying process used for liquid extract has a higher yield. The determination of dry residue in fluid extracts aims to define total solids of the extractive solution [25]. The value obtained means that for each 100g of extractive solution prepared as detailed above, 3.0984 g of soluble solids are obtained. Thus, by determining dry residue it is possible to predict the yield of the dried product.

Phytochemical prospection of plants is important to know the chemical compounds present in plant species and to identify secondary metabolite groups that are relevant for health [26]. Hydrolyzable tannins, condensed tannins, flavonoids, steroids and saponins were investigated, as these metabolites were described as present in leaves, stem, fruits and seeds *of L. ferrea*. Table 3 shows the results of Thin Layer Chromatography (TLC) for each metabolic group surveyed.

Table 3: Secondary metabolites present in *L. ferrea* seed

Metabolic groups	Presence or absence of metabolites
Hydrolysable tannins	+
Condensed tanning	-
Flavonoids - Quercetin	+
Steroids	-
Saponins	+

Subtitle: Presence (+); Absence (-)

Through these results, the presence of hydrolysable tannins, flavonoid (quercetin) and saponins was confirmed in the plant seed, corroborating with previous studies for *L. ferrea*, in which the secondary compounds were condensed and hydrolysable tannins, coumarins, flavonoids, saponins and steroids [27].

In addition to the qualitative tests, the total flavonoid content (TFC) was obtained expressed as a percentage (% w/w), calculated as quercetin values [16]. The total flavonoid content for the ethanolic extract of *L. ferrea* seeds was 31.00 ± 0.004 mgEQ/100g (milligram of quercetin equivalent per 100g of the extract). It is worth noting that this value may be subject to some variations, as the levels of secondary metabolites of plants depend on several factors such as: place of collection, seasonality, temperature, and plagues. [28].

After the drying procedure to obtain the dry extract of *L. ferrea* seeds, the process resulted in a yield of approximately 90% and the residual humidity was 3.95 ± 0.56%. These results are in accordance with the Second Supplement of the Brazilian Pharmacopoeia 6th edition [14] for *L. ferrea*, which states that the residual moisture should be a maximum of 5%.

Regarding the ointment, formulation tests were important in determining the best excipient concentration that would exhibit the best extract

incorporation, viscosity and spreadability. The BL III was the one that best fit the desired characteristics.

In vitro antimicrobial activities were measured in terms of inhibition halos (mm) in which arithmetic means were recorded in Table 4 for the dry extract and Table 5 for the extract-based ointment.

Table 4: Inhibition halos of dry extract of *L. ferrea* seeds against standard microorganisms.

Microorganisms	Inhibition halos (mm)			
	75.00 %	37.20 %	18.70 %	9.40 %
Staphylococcus aureus	198.66	183.61	159.53	81.27
Escherichia coli	93.15	81.13	72.12	66.11
Klebsiella pneumoniae	21.00	20.25	18.00	1.50
Pseudomonas aeruginosa	234.97	216.90	198.82	180.75
Candida albicans	64.37	58.11	51.11	32.82

Table 5: Inhibition halos of the ointment based on dry extract of *L. ferrea* seeds against standard microorganisms.

Microorganisms	Inhibition halos (mm)			
	75.00 %	37.20 %	18.70 %	9.40 %
Staphylococcus aureus	33.0	30.5	26.5	13.5
Escherichia coli	15.5	13.5	12.0	11.0
Klebsiella pneumoniae	14.0	13.5	12.0	-
Pseudomonas aeruginosa	19.5	18.0	16.5	15.0
Candida albicans	33.0	30.5	26.5	13.5

The results obtained from the study of antimicrobial activity showed that the dry extract of *L. ferrea* seeds and ointment based on 75% of the extract showed a concentration-dependent inhibitory effect, in which the largest zone of inhibition was against *Candida albicans*, being the most sensitive microorganism, followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. The Minimum Inhibitory Concentrations (MIC) presented by the dry extract and formulation are shown in Table 6.

Table 6: Minimum Inhibitory Concentration of dry extract of *L. ferrea* seeds and ointment based on 75% of extract.

Microorganisms	MIC (%)	
	EXTRACT	OINTMENT
Candida albicans	0.39	0.58
Staphylococcus aureus	1.56	9.4
Klebsiella pneumoniae	6.25	9.4
Pseudomonas aeruginosa	0.78	9.4
Escherichia coli	3.12	18.75

Staphylococcus aureus and *Pseudomonas aeruginosa* are responsible for causing ophthalmic infections and belong to the list of most frequently agents found in clinical isolates [29, 30], also present in dermatological infections [31, 32]. *Klebsiella pneumoniae* are responsible for a wide variety of infections, including pneumonia, urinary tract infections and liver abscesses [33], *Escherichia coli* is not usually mentioned in ophthalmic infections, however it is one of the causative agents of dermatological infections [29, 34]. Potentially fatal diseases, such as mycoses caused by opportunistic fungal pathogens (mainly Candida species) associated with nosocomial infections, are a major health problem today [3].

Activity against *Staphylococcus aureus* is of significant interest because it is commonly found on hands, face and deep layers of the skin and it is perhaps the most commonly found, and difficult to eliminate, especially in the deepest layers of the skin [35, 36].

Phenolic compounds, usually present in plant extracts, may be involved in their antimicrobial activity. Some researchers have shown that antimicrobial activity is proportional to the amount of phenolic compounds in the plant [1, 3].

The ointment based on the dry extract of *L. ferrea* seeds was very effective against all evaluated microorganisms (Gram positive bacteria, Gram negative bacteria, and yeast). After investigation of the secondary metabolites present in *L. ferrea* seeds it was possible to find, among other compounds, tannins, flavonoids and saponins. According to Harkat-Madouri et al. [37] and Sagbo et al. [38], flavonoids and tannins are responsible for several

biological activities, including antimicrobial properties. Del Hierro et al. [39] and Guo et al. [40] state that saponins are secondary compounds that also have antimicrobial activity.

According to Cushnie and Lamb [41], preparations containing flavonoids and their constituents were used by physicians and healers as an alternative in the treatment of several human diseases. An example is the use of propolis in the treatment of wounds and ulcers by Hippocrates in ancient Greece due to its healing properties. The antimicrobial effect of flavonoids is correlated to phenolic hydroxyl groups that exhibit protein affinity and therefore act as inhibitors of bacterial enzymes, interfering with their synthesis pathways [42].

The antimicrobial activity is one of countless scientifically proven pharmacological actions of *L. ferrea*. According to Sampaio et al. [9], *L. ferrea* fruits have antimicrobial activity against oral pathogens such as *Candida albicans*, *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus oralis*, and *Lactobacillus casei*. According to the study by Oliveira et al. [43], a significant antimicrobial activity was observed in an ethanolic extract of *L. ferrea* against most of the tested microorganisms, in which it presented activity superior to the positive control for *Staphylococcus* sp. It has also been proven in other extracts from diferent parts of the plant [44].

Studies by Paiva et al. [45] demonstrated that crude hydroalcoholic extract of *L. ferrea* peel has natural antimicrobial potential against caprine mastitis caused by microorganisms. Another important antimicrobial property that is being studied is the use of *L. ferrea* in the treatment of biofilms formation. Several flavonoids have been shown to inhibit biofilm formation from *Streptococcus mutans*, *Aeromonas hydrophila*, and *Escherichia coli* [46].

Venancio et al. [47], demonstrated that *L. ferrea* extract has good efficacy against oral cavity pathogens, as well as against *S. mutans* and *S. oralis* strains by microdilution tests for determination of Minimum Inhibitory Concentration (MIC).

Similar properties have been researched in other species of the same family of *Caesalpinia cacalaco* in which their antimicrobial activity has been proven in different concentrations of phenolic compounds extracted from seed [48] and *Caesalpinia coriaria*, that through biosynthesis and characterization of silver metal nanoparticles using leaf extracts, showed significant antimicrobial efficacy [49].

The results found in this work suggest that the ointment based on the ethanolic extract of *L. ferrea* seeds has antimicrobial activity against microorganisms of clinical interest, after using *in vitro* models, through bacteriostatic action. These results are promising, as microbial infections continue to be a serious health problem worldwide [50] and since microbial resistance to the conventional antibiotics used in the therapy is becoming more common. In this sense, the discovery of new alternatives, especially natural ones, that are active against the broader spectrum of Gram positive, Gram negative and Candida species is a promising and urgent research area [3,51].

5. CONCLUSION:

The physicochemical characterization of the ethanolic extract of the *Libibidia ferrea* seed should be considered to establish the standardization of plant raw material, as well as to guarantee the material quality, reproducibility, and safety of the plant material.

Through the development of the ointment based on the ethanolic extract of *L. ferrea* seeds, the BL III presented the best pharmacotechnical characteristics. The dry extract of *L. ferrea* seeds and ointment based on 75% of the extract showed a concentration-dependent inhibitory effect. Increased extract concentration did not affect the pharmacotechnical characteristics of the formulation. In the determination of microbiological activity, the ointment inhibited the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*, which are responsible for several microbial infections and represent a public health problem. As future perspectives, this work aims to optimize and bring more innovations to the formulation, as well as to carry out experiments in vivo so that the activity of the extract can be safely confirmed.

CONFLICTS OF INTEREST

The authors declare no conflict of interest that could constitute an impediment to the publication of this article.

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