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***Libidibia ferrea* antimicrobial and disinfectant activity**

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ABSTRACT

Microorganisms are becoming resistant to the commonly used chemical disinfectants. Thereby, these chemical products should now be replaced by natural ones. In this context, this research aimed to evaluate the disinfectant activity of the extract and decoction of *Libidibia ferrea* leaves in different surfaces. Thus, two forms of jucá leaf extraction (decoction and hydroalcoholic extraction) were tested through the diffusion disk technique from Kirby and Bauer, growth curve, acceptability test with the population, and surface test. Results showed sensibility to decoction of *S. aureus* and *E. faecalis* strains; however, for the extract, the strains that demonstrated sensibility were *S. aureus*, *P. aeruginosa*, *Micrococcus spp.*, *Corynebacterium spp.*, and *S. Typhimurium* in the diffusion disk test. A reduction of the bacterial charge throughout the growth curve was seen through the absorbance values after 24 hours to the strains of *E. coli*, *E. faecalis*, *Micrococcus spp.* and *K. pneumoniae* in the concentration of 100 mg/mL of the decoction and extract. Regarding the surfaces test, there was a reduction in the bacterial charge in all tested strains. Therefore, the *L. ferrea* may be used as an effective alternative disinfectant measure.

Keywords: Fabaceae; jucá; plants, medicinal; plant extracts.

INTRODUCTION

Microorganisms, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, can adapt to different environments and be easily spread by humans through food, and they can also be distributed in the environment, air, dust, and sewage water. In general, microorganisms are becoming resistant to the commonly used chemical disinfectants, either by its use and incorrect destination or by the formation of biofilms or resistance transfer (DOLAN; COSTERTON, 2002).

Therefore, these products should now be replaced by natural ones, which present a lower degree of toxicity, being more economically viable and more accessible to the population (VOLKART; SPAGIARI; BIZZANI, 2017; NÓBREGA; DANTAS; SILVA, 2010). Among the species of used plants is the *Libidibia ferrea* (*Febaceae*) that is natural of the Caatinga biome and has been described from the Northeast to the South of Brazil. It is popularly known as Pau-ferro, Pau-caí or Jucá (MOTA; FERREIRA; IMAÑA, 2012). The *Libidibia ferrea* has antifungal, antibacterial, antiulcerogenic, anti-inflammatory, antidiabetic, anti-flu, antitussive, anti-asthmatic, antianemia, antidiarrheal, anticoagulant, analgesic, and healing actions. It is also a larvicide against the *Aedes aegypti*, and it is indicated for feeding animals as in sheep and goat farming, widely used in rural areas, given the difficulty of food (GOMES et al., 2017; MOREIRA; OLIVEIRA, 2017; REIS et al., 2017; PEREIRA et al., 2016; HASSAN et al., 2015; FREITAS et al., 2015; MAGALHÃES et al., 2014; VÁSQUEZ; MENDONÇA; NODA, 2014; LIMA et al., 2012; VASCONCELOS et al., 2011).

Despite the proven action of *Libidibia ferrea*, more studies that explore its use as a disinfectant for surfaces is required in this context. Thus, this research aimed at evaluating the disinfectant activity of the extract and decoction of *Libidibia ferrea* leaves in different surfaces.

MATERIALS AND METHODS

Microbiological analyzes and decoction production of *Libidibia ferrea* leaves were carried out at the Veterinary Microbiology Laboratory (LAMIV) of Universidade Federal do Semi-Árido (UFERSA). The analyses of phytochemical compounds and extract hydroalcoholic production of *Libidibia ferrea* leaves were performed at the Chromatography Laboratory of Universidade Estadual do Rio Grande do Norte (UERN).

Microorganisms isolated from the environment (*Staphylococcus aureus*, *Micrococcus* spp., *Corynebacterium* spp. – LAMIV) and standard strains (*Staphylococcus aureus* – American Type Culture Collection – ATCC 25923, *Escherichia coli* – ATCC 25922, *Enterococcus faecalis* – ATCC 29212, *Pseudomonas aeruginosa* – ATCC 27853, *Streptococcus agalactiae* – ATCC 13813, *Salmonella Typhimurium* – ATCC 14028, *Klebsiella pneumoniae* – ATCC 700603) were used for this research.

100 g of leaves from *L. ferrea* were used for decoction and they were deposited at a container with 200 mL of distilled water. Then, they were successively boiled at water bath for 15 minutes to produce 200 mL of decoction. The produced solution was considered at the concentration of 1:2 (LEONEZ et al., 2018). In the hydroalcoholic extract production, the leaves were shredded, weighted and wrapped into a properly identified amber glass container that was immersed during seven days in a hydroalcoholic solvent solution (ethanol 70%), which was agitated every 24 hours. After the immersion time, the compound was taken to vacuum filtration, and afterwards to the evaporated in water bath at a temperature of $50 \pm ^\circ\text{C}$.

Microorganisms with the 1.5×10^8 UFC/mL concentration were equivalent to the pattern 5 of the McFarland's scale. These were cultivated in brain heart infusion (BHI) broth at 37°C . Five microdilution plates with 96 wells each were utilized (ALAMAR®, Diadema, São Paulo, Brazil). Each microorganism had its growth analyzed in triplicate at the concentrations of 100 mg/mL from the extract and from the decoction, as well with the positive control with the alcoholic solution of chlorhexidine (CP) at 0.5% and the negative control of dimethyl sulfoxide (DMSO).

The methodology proposed by Engel et al. (2017) was adapted in order to evaluate the surface disinfectant effects. Plastic, steel, and ceramics surfaces were washed and sterilized for use. Afterwards, the surfaces were left in contact with the inoculum in a concentration of approximately 10^8 UFC/mL during 30 minutes for biofilm formation. Posteriorly, the disinfectant solutions were sprayed at 100 mg/mL and control product, for 15 minutes, in an area of 1 cm^3 . Then, the samples were collected with a sterilized swab, and the number of microorganisms was quantified by the plate count technique (TORTORA; FUNKE; CASE, 2012).

50 judges belonging to the traditional rural communities of Mossoró/RN were randomly chosen to perform the acceptability test on the action of *L. ferrea*-based disinfectant at the concentration of 100 mg/mL, which was noticed in the *in vitro* test (PAGANI et al., 2015). A hedonic scale adapted from Pagani et al. (2015) and Beserra et al. (2003) was applied to evaluate the two surfaces: one with the commonly used disinfectant and the other with the disinfectant made of jucá leaves. Each judge received a file including the sensorial evaluation, with a nine-point structure, from “extremely liked” to “extremely disliked”, to each evaluated attribute (aroma, color, texture, and surface aspect).

Data were expressed in mean values \pm standard deviation as well with frequency (%) through the SigmaPlot program (Systat Software, Inc.), version 12.0. After analysis of the parametric assumptions and statistical differences between the experimental groups, in the different studied variables, there was a comparative analysis between the microorganisms and the decoction and extract, as well as between the varied concentrations applied to both the decoction and the extract, for one-way ANOVA and one-way ANOVA repeated measures (RM) followed by Turkey’s test, respectively. The nonparametric data were tested by Kruskal-Wallis and Friedman tests. Lastly, the statistical differences of the scores related to the sensorial analysis between groups were obtained by Wilcoxon’s text. Values of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Diffusion test results in wells used the decoction and the hydroalcoholic extract from *C. ferrea* leaves to the bacteria are described in Tables 1 and 2. It has been noticed that the strains of *Staphylococcus aureus* and *Enterococcus faecalis* demonstrated sensibility at the concentration of 100 mg/mL regarding the decoction of *L. ferrea* with halos of 10 and 10.67 mm, respectively. According to Thomazi, Bertolin and Pinto (2010), such values were considered satisfactorily, whereas they were sensitive for halos equal to or larger than 10 mm.

Table 1. Mean values and standard deviation of the formed halos used in the diffusion disk test in wells to the decoction of *Libidibia ferrea* leaves.

Microorganisms	Concentration					
	100	50	25	12.5	Positive Control Chlorhexidine 0.5%	DMSO
S.A. – Standard strain	10.0 ± 0.0b	-	-	-	24.5 ± 0.5Aa	-
<i>E.coli</i>	-	-	-	-	23.5 ± 1.5A	-
P.A.	-	-	-	-	17.67 ± 0.58C	-
<i>Micrococcus</i>	-	-	-	-	11.67 ± 0.58D	-
<i>Corynebacterium</i>	-	-	-	-	20.0 ± 1.0B	-
E.F.	10.67 ± 0.58ab	9.0 ± 0.0bc	6.67 ± 5.77bc	3.0 ± 5.20c	12.43 ± 0.51Da	-
<i>S. agalactiae</i>	-	-	-	-	20.67 ± 0.58B	-
<i>Salmonella</i>	-	-	-	-	16.5 ± 0.5C	-
<i>Klebsiella</i>	-	-	-	-	22.0 ± 1.0AB	-
S.A. – Environmental strain	-	-	-	-	23.33 ± 2.31AB	-

^{A,B}Averages accompanied by capital letters in the column mean statistical difference ($p < 0.05$); ^{a,b}averages accompanied by lowercase letters on the line means statistical difference ($p < 0.05$); PC: positive control; *absence of inhibition halo; DMSO: dimethyl sulfoxide.

Regarding to the results of the *L. ferrea* extract (Table 2), the strains of *Escherichia coli*, *Streptococcus agalactiae* and *Klebsiella pneumoniae* did not form satisfactory inhibition halos. The decoction utilized at the concentrations of 100, 50, 25 and 12.5 mg/mL inhibited the growth of the *E. faecalis* strains, being the highest statistical concentration similar to the

positive control. These results are justified by the cell wall composition of the Gram-positive microbial agent, which is more simplified than Gram-negative bacteria. It is noteworthy that these results are promising, since the decoction technique of *L. ferrea* is simple, decreasing the quantity of these microorganisms in surfaces of tools.

Table 2. Mean values and standard deviation of the formed halos according to the diffusion disk test in wells utilizing the hydroalcoholic extract of the *Libidibia ferrea* leaves.

Microorganisms	Concentration					
	100	50	25	12.5	Positive Control Chlorhexidine 0.5%	DMSO
S.A – Standard strain	18.33 ± 0.58Aab	17.0 ± 0.0Ab	11.67 ± 0.58Abc	8.0 ± 1.73Ac	24.5 ± 0.50Aa	-
<i>E. coli</i>	-	-	-	-	23.33 ± 1.52A	-
P.A	13.0 ± 0.0Ba	12.0 ± 1.0Ba	-	-	17.67 ± 0.58aB	-
<i>Micrococcus</i>	13.33 ± 0.58Ba	11.67 ± 0.58Ba	-	-	11.67 ± 0.58aC	-
<i>Corynebacterium</i>	14.0 ± 0.0Bb	12.33 ± 0.58Bb	-	-	20.0 ± 1.0aA	-
E.F	17.33 ± 0.58Aa	15.33 ± 1.53Aab	11.33 ± 0.58Ab	10.0 ± 1.0Ab	13.33 ± 1.53BCab	-
<i>S. agalactiae</i>	-	-	-	-	20.67 ± 0.58A	-
<i>Salmonella</i>	13.0 ± 0.0Ba	12.0 ± 0.0Ba	-	-	15.67 ± 1.53aC	-
<i>Klebsiella</i>	-	-	-	-	21.0 ± 2.0A	-
S.A – Environmental strain	11.0 ± 1.0Cb	7.67 ± 0.58Cb	-	-	23.33 ± 2.31aA	-

^{A,B}Averages accompanied by capital letters in the mean statistical difference column ($p < 0.05$); ^{a,b}averages accompanied by lowercase letters on the line mean statistical difference ($p < 0.05$); PC: positive control; *absence of inhibition halos; DMSO: dimethyl sulfoxide.

The decoction did not inhibit the strains of *Klebsiella pneumoniae*, *E. coli*, *Pseudomonas aeruginosas*, and *Salmonella Typhimurium*. These bacteria are Gram-negative and present structures at the cell wall, as the outer membrane, composed by lipids and lipopolysaccharides (TORTORA; FUNKE; CASE, 2012) that may hinder the entrance of hydrophilic molecules present in the decoction.

Table 3 includes the results of the growth curve utilizing the hydroalcoholic extract and the decoction of *L. ferrea* to strains that presented sensibility utilizing the best concentration

(100 mg/mL), which was noticed in the diffusion technique in wells. In the decoction, we noticed that the *E. feacalis* strain obtained a reduction in the absorbance value of 0.24. Although the *S. aureus* had its absorbance value reduced in 0.07, there was no statistical difference. Since for the extract there was a reduction in the bacterial number considering the absorbance after 24 hours, in the strains of *P. aeruginosa*, we obtained a difference in the absorbance value of 0.36. It was verified that although there was an increase in the absorbance value of *Corynebacterium* spp., *E. feacalis* and *Salmonella Typhimurium* of 0.06, 0.03, and 0.14, respectively, these did not present statistical difference as for the positive control.

Table 3. Mean values \pm standard deviation of the growth curve for the microdilution technique of the hydroalcoholic extract and the decoction of *Libidibia ferrea* leaves to the microorganisms in the dilution of 100 mg/mL in 24 hours.

Microorganisms	Groups	0h	24h
S.A. – standard strain	Extract	1.46 \pm 0.27bA	1.70 \pm 0.10aA
	Decoction	0.48 \pm 0.0aB	0.41 \pm 0.12aB
<i>E. coli</i>	Extract	1.20 \pm 0.06aA	1.19 \pm 0.04aA
	Decoction	0.56 \pm 0.01aB	0.42 \pm 0.09bB
P.A.	Decoction	1.43 \pm 0.29aA	1.07 \pm 0.07bA
	Extract	0.65 \pm 0.06aB	1.31 \pm 0.19bA
<i>Micrococcus</i>	Extract	1.53 \pm 0.15bA	1.76 \pm 0.10aA
	Decoction	0.61 \pm 0.03aB	0.36 \pm 0.02bB
<i>Corynebacterium</i>	Decoction	1.18 \pm 0.20aA	1.24 \pm 0.20aA
	Extract	0.72 \pm 0.21aA	0.68 \pm 0.11aB
E.F.	Extract	1.33 \pm 0.21aA	1.39 \pm 0.21aA
	Decoction	0.59 \pm 0.12aB	0.35 \pm 0.02bB
<i>S. agalactiae</i>	Decoction	1.55 \pm 0.36aA	1.33 \pm 0.15bA
	Extract	0.48 \pm 0.03aB	0.46 \pm 0.05aA
S.T.	Extract	1.55 \pm 0.36bA	1.69 \pm 0.41aA
	Decoction	0.52 \pm 0.01aA	0.41 \pm 0.13aB
<i>Klebsiella</i>	Decoction	1.18 \pm 0.19aA	1.26 \pm 0.05aA
	Extract	0.55 \pm 0.02aB	0.28 \pm 0.22bB
	Decoction		

S.A. – environmental strain	Extract	1.05 ± 0.17bA	1.20 ± 0.13aA
	Decoction	0.37 ± 0.03aB	0.40 ± 0.11aB

^{A,B}Averages accompanied by capital letters in the mean statistical difference column ($p < 0.05$ — Mann-Whitney); ^{a,b}averages accompanied by lowercase letters on the line means statistical difference ($p < 0.05$ — Wilcoxon); *it was not tested because there were no results in the diffusion disk test.

The results indicate that the hydroalcoholic extract presented a more efficient effect than that presented by the decoction, since the absorbance values were lower after 24 hours and it has inhibited the development and growth of microorganisms as *P. aeruginosas* and *Salmonella Typhimurium*. When comparing the results between the microorganisms, it may be observed a lower bacterial charge between themselves, presenting a statistical difference.

Such variation may occur due to the different forms of extraction, since the extract may have molecules that can penetrate the outer membrane of the Gram-negative bacteria cell wall, but not in Gram-positive ones (DUFFY; POWER, 2001). Or yet, it can happen due to phytochemical compounds in the extract that presented a higher bioactivity on Gram-negative bacteria, because of a higher affinity from these by its membrane lipid structure that surrounds them, as evidenced by Engel et al. (2017), where the utilized strains of *Salmonella* were more sensible than the strains of *S. aureus* both for the disinfectant containing carvacrol and for the disinfectant containing timol.

A reduction of UFC/mL/cm² was observed considering all tested microorganisms (Table 4). As a result, in all tested strains, there was bacterial reduction of the extract and decoction with statistical difference, considering the negative control, in all surfaces, with the exception of the *Salmonella Typhimurium* in relation to the steel. Therefore, it was observed that the related data are according to Oliveira et al. (2017). In order to have a good disinfectant it is necessary that it has the capacity to destroy or to inactivate the pathogenic organisms to be eliminated, in a reasonable time, with a good cost/benefit, presenting facility and safety in its transport, storage, handling, and application.

Table 4. Mean values of the bacterial number according to the hydroalcoholic extract based disinfectant and the decoction of the *Libidibia ferrea* leaves in steel, plastic, and ceramics surfaces.

Microorganisms (UFC x 10 ⁸)	Groups	PC	NC	Extract	Decoction
<i>Staphylococcus aureus</i>	Steel	-*	0.564x10 ⁵ Bb	0.39x10 ⁶ Aa	0.22x10 ⁶ Aa b
	Plastic	0.3x10 ⁵ c	0.243x10 ⁶ Aa	0.124x10 ⁶ A b	0.39x10 ⁵ Bc
	Ceramics	-*	0.176x10 ⁷ Aa	0.35x10 ⁵ Bb	0.31x10 ⁵ Bb
<i>Pseudomonas aeruginosa</i>	Steel	0.16x10 ⁴ Ac	0.144x10 ⁶ Aa	0.51x10 ⁵ Ab	-**
	Plastic	-*	0.83x10 ⁵ Ba	0.38x10 ⁵ Ab	-**
	Ceramics	0.12x10 ⁴ Ab	0.378x10 ⁵ Ba	0.58x10 ⁵ Aa	-**
<i>Micrococcus</i> spp.	Steel	0.147x10 ⁴ A a	0.657x10 ⁴ Ba	0.43x10 ⁴ Aa	-**
	Plastic	0.37x10 ³ Ab	0.87x10 ⁴ Ba	0.97x10 ³ Bb	-**
	Ceramics	0.47x10 ³ Ab	0.126x10 ⁵ Aa	0.41x10 ⁴ Ab	-**
<i>Corynebacterium</i> spp.	Steel	0.33x10 ³ Ab	0.21x10 ⁵ Ba	0.197x10 ⁴ A b	-**
	Plastic	0.173x10 ⁴ A b	0.22x10 ⁶ Aa	0.2x10 ⁴ Ab	-**
	Ceramics	0.3x10 ² Ac	0.149x10 ⁶ Aa	0.113x10 ⁴ A b	-**
<i>Enterococcus faecalis</i>	Steel	-*	0.251x10 ⁵ Ba	0.57x10 ³ Ac	0.28x10 ⁴ A b
	Plastic	0.3x10 ² b	0.13x10 ⁵ Aa	0.17x10 ³ Ab	0.17x10 ³ Bb
	Ceramics	-*	0.727x10 ⁴ Ba	0.7x10 ² Ab	0.4x10 ³ Bb
<i>Salmonella Typhimurium</i>	Steel	0.23x10 ³ Ac	0.162x10 ⁶ Aa	0.239x10 ⁵ A b	-**
	Plastic	0.11x10 ⁴ Ab	0.5x10 ⁴ Bb	0.647x10 ⁵ A a	-**
	Ceramics	-*	0.256x10 ⁵ A Ba	0.767x10 ⁴ B b	-**
<i>Staphylococcus aureus</i>	Steel	0.7x10 ² Ac	0.293x10 ⁶ Aa	0.6x10 ³ Ab	-**
	Plastic	0.37x10 ³ Ab	0.303x10 ⁴ Ba	0.47x10 ³ Ab	-**
	Ceramics	0.7x10 ² Aa	0.2x10 ³ Ca	0.17x10 ³ Aa	-**

^{A,B}Averages accompanied by capital letters in the column means statistical difference ($p < 0.05$); ^{a,b}averages accompanied by lowercase letters on the line means statistical difference ($p < 0.05$); the results are in UFC/mL/cm²; *reading in the value of 0; **it was not tested on the decoction; PC: positive control; NC: negative control.

The results, according to the variables smell, color, texture, surface aspect (based on its cleanliness and brightness), utilized to evaluate the acceptability of the disinfectant are described in Table 5. In all the evaluated criteria there was a higher acceptability of the disinfectant made from *L. ferrea* leaves, with a higher frequency of acceptance (83.6%) in the aroma. There is no report of natural products utilized as disinfectants, regarding acceptability. However, Teixeira and Becker (2017) claim that the best product for disinfection and sanitation in surfaces is the 70% alcohol, since it is so effective as sodium hypochlorite, with a contact of at least 10 minutes.

Table 5. Mean values \pm standard deviation (SD) and accumulated frequency (%) of the notes between 6 and 9 assigned to the acceptability criteria of the utilized disinfectants.

Variables	Experimental groups				p-value
	Jucá disinfectant		Common disinfectant		
	Mean \pm SD	%	Mean \pm SD	%	
Aroma	7.33 \pm 1.52	83.6	4.36 \pm 2.05	27.3	<0.001*
Color	5.44 \pm 1.18	36.4	4.64 \pm 1.41	23.6	<0.001*
Texture	6.35 \pm 1.21	74.5	4.91 \pm 1.55	30.9	<0.001*
Surface aspect	6.95 \pm 1.38	81.8	4.67 \pm 1.72	25.5	<0.001*

*Statistical difference ($p < 0.05$ — Wilcoxon); SD: standard deviation.

CONCLUSION

The decoction and extract of *L. ferrea* may be utilized as an efficient alternative of disinfectant measurement, because it has antimicrobial activity in Gram-positive and negative strains. It can also be used due to its reduction of the bacterial charge in the tested surfaces and its excellent acceptability by the audience.

ETHICS AND BIOSAFETY COMMITTEE

It was submitted and consequently approved by the *Comitê em Ética e Pesquisa da Universidade Estadual do Rio Grande do Norte* under CAAE: 03621718.0.0000.5294 and protocol number: 3.147.117.

AUTHORS' CONTRIBUTIONS

Francisco Marlon Caneiro Feijó and Nilza Dutra Alves created the research project and monitored all the research stages. Jamile Rodrigues Cosme de Holanda, Caio Sérgio Santos and Gardênia Silvana de Oliveira Rodrigues set up the experiments and collected the data. After data collection, Wesley Adson Costa Coelho and Jamile Rodrigues Cosme de Holanda analyzed the data and prepared the entire article. All authors read and approved the final article.

CONFLICT OF INTERESTS

The authors declare there is no conflict of interests.

FINANCING

None.

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