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Original Article

ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITIES OF *LIBIDIBIA FERREA* BARK AND FRUIT EXTRACTS

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ABSTRACT

Objective: The aim of this study was to investigate the antioxidant and hepatoprotective activities of *Libidibia ferrea* bark and fruit extracts.

Methods: The extracts were analyzed by high performance liquid chromatography. Antioxidant potential was studied using DPPH, ABTS, superoxide anion radical, β-carotene and cellular assay. The extracts were also evaluated for hepatoprotective activity against carbon tetrachloride (CCl_4) induced acute liver injury in mice.

Results: Both fruit and bark extracts demonstrated high free radical scavenger activities using DPPH or ABTS assays and superoxide anion radical tests when compared with standards. Extracts of *L. ferrea* also demonstrated antioxidant activity in a cell-based assay. In this assay, bark extract showed better activity than fruit extract (more than 60% inhibitions at extract concentration 50 µg/mL). Extracts significantly prevented the CCl_4 induced hepatic damage as indicated by serum marker enzymes (GOT, GPT and ALP). *L. ferrea* extracts also prevented CCl_4 induced oxidative stress by inhibiting lipid peroxidation. Both bark and fruit extracts showed substantial antioxidant activity explained by the presence of polyphenol compounds like gallic and epicatechin. The administration of bark and fruit extracts from *L. ferrea* prevented liver biochemical alteration induced by CCl_4.

Conclusion: The extracts of *L. ferrea* possess free radical scavenging activity under *in vitro* conditions and cell-based assay, and could protect the liver against CCl_4 induced oxidative stress by increasing antioxidant defense activities.

Keywords: *Libidibia ferrea*, Antioxidant, Hepatoprotective activity.

INTRODUCTION

Antioxidants are compounds that protect organisms against oxidative damage, which is involved in several pathologies such as rheumatoid arthritis, cancer, arteriosclerosis and Alzheimer's disease [1-3]. Antioxidant defenses include catalytic removal of free radicals and reactive species by enzymes (catalase, superoxide dismutase, peroxidase) and thio-specific antioxidants and by binding proteins to pro-oxidant metal ions (e. g., copper and iron). Antioxidants can also provide protection against macromolecular damage by proteins such as stress or heat shock proteins and reduction of free radicals by electron donors (uric acid, bilirubin, GSH, vitamin C, and vitamin E) [4].

The liver plays a critical role in regulating several important functions including synthesis, secretion, and xenobiotic metabolism [5]. The liver produces large amounts of reactive oxygen species (ROS) in the course of detoxifying xenobiotic and toxic substances, and oxidative stress caused by ROS has been shown to be linked to liver diseases, such as steatosis, and other pathological liver conditions [6]. Liver damage is a widespread pathology, which can influence these physiochemical functions and be caused by viral hepatitis, alcoholism, or liver-toxic chemicals, such as carbon tetrachloride (CCl_4). CCl_4 is a xenobiotic reported to induce acute and chronic tissue injuries *in vitro* and induce an increase in lipoperoxides and free peroxide radical concentrations that are highly reactive and cause injury or necrosis.

Herbal products have been widely used for protection against chemical-induced toxicities because of their safety and efficacy. Herbal extracts have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. A number of studies have shown that herbal extracts possess antioxidant activity against CCl_4 hepatotoxicity by reducing oxidative stress and inhibiting lipid peroxidation [7]. *L. ferrea* Mart. (FABACEAE) is a plant that grows throughout Brazil and is widely used in traditional medicine for treating several diseases such as diabetes, hypertension, enterocolitis and atherosclerosis. Pharmacological studies have described its anti-inflammatory [8], cancer chemopreventive [9], cardiovascular [10] and antimicrobial properties [11]. Phytochemical studies demonstrate the presence of flavonoids, tannins and other phenolic compounds, mainly gallic acid and ellagic acid [9,12] classes of substances already mentioned in the literature as potential antioxidants are present in *L. ferrea* extracts. Thus, in order to contribute further to the knowledge of *L. ferrea* as a medicinal plant, this vegetable species was screened to determine its antioxidant and hepatoprotective activity.

MATERIALS AND METHODS

Plant material

Dry fruits and bark of *L. ferrea* Mart. were collected in the city of Manaus, in the state of Amazonas, Brazil, in August 2007, and identified in the Herbarium of Instituto Nacional de Pesquisas da Amazonia (INPA), voucher number 802222. The seeds were withdrawn from desiccated fruit. The fruits without seeds and barks were dried for seven days in a circulating-air oven at 40° ± 5°C temperature. After drying, the material was sliced and separated by a knife mill (1 mm mesh) and stored in polystyrene bottles.

Preparation of spray-dried extract

The spray-dried extract from both plant material (dry fruits and bark) were obtained from extractive solutions dried in a spray-dryer apparatus (MD-01, Labmaq, Brazil). The extractive solution, from dry fruits and bark of *L. ferrea*, were obtained through infusion method using 7.5% plant material (w/V), boiling water as a solvent and 15 minutes of extraction.[13,14]

Chromatography profile

The analyses were carried out using a Shimadzu liquid chromatography device equipped with a pump (LC-10 AD), an automated gradient controller (FCV-10 AL), an auto sampler (SIL-10

A) and a UV/VIS detector (SPD-10A). The equipment was controlled by Class LC-10 software. The analytical column was a RP-18 Gemini 250 x 4 mm i. d., 5-µm particle diameter (Phenomenex, USA). A pre-column Phenomenex loaded with the same material was employed to protect the analytical column. The chromatography profile was performed using as a mobile phase with acetic acid 0.5 % (w/w) as solvent A and acetonitrile: acetic acid 0.5 % (w/w) (50:50 (v/v)) as solvent B at a flow-rate of 0.8 ml. min^{-1}. The gradient program was as follows: 12.5 - 20 % B (5 min), 20 - 40 % B (16 min), 40 – 80 % B (9 min), 80 B % (5 min), 80 – 100 % B (5 min), 100 – 12.5 % B (15 min). The injection volume for all samples was 20 µl. The peaks were detected at 275 nm and 350 nm.

Antioxidant Activity

DPPH method

The assay employed was based on methods from the literature [15] with small adaptations that allowed tests to be run in 96-well plates. Initially, DPPH solution (0.8 mmol/L in EtOH) was prepared. The final volume of each test well (350 µL) was composed of extract dissolved in EtOH (250 µL), or pure EtOH in the case of controls, and 0.8 mM DPPH solution (100 µL). An initial spectrophotometric reading of each extract in EtOH was performed (blank, Abs_1) using a test plate reader (TP-Reader, Thermoplate, Italy). After addition of DPPH solution to wells, the test plate was allowed to stand in the dark at room temperature for 30 min and then the absorbance was measured at 517 nm (Abs_2). Ascorbic acid (vitamin C) and Gallic acid were used as antioxidant standard. The results were obtained using the following formula:

$$\% \text{ Inhibition} = 100 \times [1 - (Abs_2 \text{ sample} - Abs_1 \text{ sample})/Abs \text{ control}]$$

ABTS method

The method is based on the oxidation of ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonate)], which was performed according to the literature [16]. Succinctly, an oxidized ABTS ($ABTS^{+}$) solution was obtained by dissolving ABTS (10 mg) in H_2O (5 mL) then adding to a 5 mM $K_2S_2O_8$ solution and allowing to stand 24 h prior to use. The assay was performed in 96-well test plates. Each well was charged with $ABTS^{+}$ solution (40 µL), deionized H_2O (60 µL) and plant extract (250 µL) in deionized H_2O at concentrations of 0.1-10 g/L or pure deionized H_2O for control wells. Initially, an absorbance reading was performed in each well containing only extract dissolved in deionized H_2O as a blank (Abs_1) 714 nm in a test plate reader (TP-Reader, Thermoplate, Italy). After addition of $ABTS^{+}$ solution, the plate was allowed to stand at room temperature and ambient light for 15 min, and then a final reading (Abs_2) was performed at 714 nm. Ascorbic acid (vitamin C) and Gallic acid were used as antioxidant standard. Antioxidant activity of extracts was calculated using the following formula:

$$\% \text{ Inhibition} = 100 \text{ x } [1 - (Abs_2 \text{ sample} - Abs_1 \text{ sample})/Abs \text{ control}]$$

Superoxide anion radical scavenger activity

The assay employed was based on method reported in the literature [17]. Succinctly, test solutions were prepared by dissolving each dry extract in ethanol (10 mg/mL), and the buffer TRIS-HCl 16 mM and pH 8.0 was used as a solvent. The wells of a test plate were charged with an extract sample (50 µL), 250 µM of nitroblue tetrazolium (NBT) (100 µL) and 390 µM NADH (100 µL). Absorbencies were recorded in a test plate reader (TP-Reader, Thermoplate, Italy) at 560 nm (Abs_1). Then, 10µM PMS (100µL) was added, and the plate was left standing for 5 min at 25 ºC. Next, absorbencies were again registered (Abs_2). Ascorbic acid (vitamin C) and Gallic acid were used as antioxidant standard. Inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = 100 \text{ x } [1 - (Abs_2 \text{ sample} - Abs_1 \text{ sample})/Abs \text{ control}]$$

Determination of the antioxidant activity with the β -carotene bleaching method

The antioxidant activity of aqueous extract of the bark and fruits of *L. ferrea* was evaluated using the β-carotene-linoleic acid model system following the method described in the literature [18] with some modification. One hundred Fifty microlitres of β-carotene (2.0 mg in 1 mL of chloroform) was added to 50 µL of linoleic acid, 200 mg of Tween 80 emulsifier mixture and 500 µL of chloroform in an Erlenmeyer flask. After evaporation of chloroform under nitrogen gas, 25 mL of distilled water saturated with oxygen were added by vigorous shaking for 30 minutes. Using 96-well microplates, 240 microlitres of this mixture were transferred into each well containing 10 µL of the extracts, water (Control) or BHT as standard. The samples were diluted (1:2) eight times (200-1.56 µg/mL). As soon as the emulsion was added to each well, the zero time absorbance was measured at 492 nm using a microplate reader Multimode Detector DTX-800 (Beckman-Coulter, Fullerton, CA, USA). The emulsion system was incubated for 2 h at 50ºC and the absorbance was measured every 15 minutes.

Cell antioxidant activity and viability test

Cell culture

Cells were grown in DMEM medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 mg/mL streptomycin and 100 U/mL penicillin and incubated at 37 ºC with a 5% CO_2 atmosphere.

Alamar blue assay

The Alamar Blue Assay was performed according to the method described in the literature [19]. NIH-3T3 cells were seeded at a density of 1 x 10^4 in 100 µL of DMEM per well in a 96-well microplate. Twenty-four hours after seeding, the cells were treated with 100 µL of the extracts (250-1.95 µg/ml) for 24 h. Doxorubicin was used as positive control (25-0.19 µg/mL) and PBS was used as negative control. After the treatment period, 10 µL of Alamar Blue (0.02%) was added to each well for 3 h. Then, the fluorescence was measured in an ELISA reader.

Cell antioxidant activity

This test was performed according to the method described in the literature [20]. NIH-3T3 cells were seeded in 96-well microplates at a density of 6 x 10^4 in 100 µL of DMEM per well. External wells were not used since they undergo more variation during the reading than inner wells. Twenty-four hours after cultivation, the culture medium was removed and the cells were washed with 100 µL of PBS, followed by a treatment for 1 h in triplicate wells by diluting the extract at concentrations of 50 µg/mL to 1.56 µg/mL. Quercetin was used as positive control at concentrations of 10 µg/mL to 0.31 µg/mL and PBS was used as a negative control. After one hour of treatment, the medium was removed and the cells were washed again with phosphate buffer, the buffer was removed and 100 mL of 2,2-azobis-2-methyl- propanediamine, dihydrochloride (AAPH) were added and immediately the microplate reader began reading at 485 nm with excitation and emission 535 nm every 10 minutes to 60 minutes. The control wells contained cells treated with AAPH and dichlorofluorescein and blank wells contained cells treated with dichlorofluorescein without AAPH. Concentrations of all samples were subtracted from the value of fluorescence of white light on their time. The antioxidant potential was expressed in fluorescence units. The fluorescence was measured in an ELISA reader (emission filter at 538 nm with excitation at 485 nm) every 10 min for 1 h.

Assessment of hepatoprotective effect

Hepatoprotective assay

Eight-week male BALB/c mice, (25-30g), obtained from the central bioterium of the Federal University of Amazonas (UFAM), were randomly divided into groups of five mice each. Groups of mice were fed a regular diet for 2 weeks, while another group of mice was fed a regular diet supplemented with extracts from *L. ferrea* at doses of 100, 200 mg/kg/w or quercetin (50 mg/kg/w) for 3 days. On the 4th day, after 4h of fasting, all mice received an intraperitoneal injection of saline or CCl_4 (2 mL/Kg body weight [BW] of 25% solution in olive oil). The mice were sacrificed 20h after the CCl_4 treatment under anesthesia with pentobarbital sodium (65mg/Kg BW). Blood samples were collected and centrifuged at 800g for 10 min to obtain serum. Livers were also excised. All the procedures were conducted

according to the "Ethical Guidelines for Laboratory Animals" prescribed by UFAM (Federal University of Amazonas).

Biochemical Assays

Biochemical parameters, such as the activities of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) were determined using COBAS MIRA PLUS® Analyser and Labtest® kits.

Determination of Malondialdehyde (MDA)

The determination of TBARS tissue was performed following the method described in the literature [21] with modifications. The liver homogenate was prepared in a proportion 1:10 (w/v), in which liver (1 g) was weighed and excised. The excised liver was added into a test tube containing 10 mL HCl 150 µM and homogenized. The TBA-TCA solution was prepared by dissolving 20 mM TBA in 10% TCA solution in HCl 0.25 M. The liver homogenate (1 mL) prepared as above was placed into a disposable test tube (10 mL) and 2 mL of the TBA-TCA solution were added. The mixture was vortexed, heated in a 90°C water bath for 15 min, cooled in a cold water bath for 10 min and centrifuged at 2,000× g for 15 min. The absorbance of supernatant was measured at 531 nm with a microsample spectrophotometer.

Statistical Analysis

Values are given as arithmetic means ± standard error of the mean (S. E. M). Data was statistically analysed using one-way analysis of variance (ANOVA) followed by Student's *t*-test.

RESULTS

The isolation of the active components from the EtOAc extract by successive chromatographies on HPLC provided two compounds that were identified as gallic acid and epicatechin by retention time and spectral comparisons of respective standards. The chromatography profile from extracts of fruits and bark of *L. ferrea* are shown in **Fig 1**. The compounds were identified through comparison of diode array (DAD) spectra and co-chromatography as standard substances. Peak 1 with a retention time of 6.6 min as gallic acid and Peak 2 with a retention time of 16.8 min as epicatechin.

Both fruit and bark extracts of *L. ferrea* demonstrated high free radical scavenger activities using DPPH. ABTS and superoxide anion radical tests (**Table 1**) compared with standards (ascorbic acid and gallic acid). Fruit and bark extracts of *L. ferrea* showed 50% inhibitory concentration (IC_{50}) value of 7.8±1.2 (fruits) and 7.8±0.9 (bark), while the standard ascorbic acid showed IC_{50} value of 2.7±0,3 and gallic acid showed IC_{50} value 1.1±0.2. Scavenging activity of radical anion superoxide examined at different concentrations of extracts of *L. ferrea* was 24.6±4.9 (extract fruit) and 31.7±2.4 (extract bark), while the standard ascorbic acid showed 6.9±3.3 and gallic acid showed 2.3±1.4.

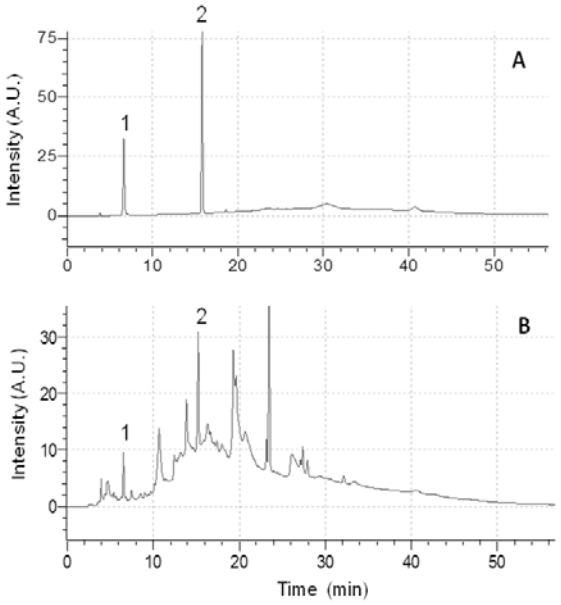

Fig. 1: HPLC profile of extract from *L. ferrea.*

In determining antioxidant activity by β-carotene/linoleic acid system, the bark extract had less discoloration than the fruit extract in the concentrations tested, which suggests that this extract has better antioxidant activity than the fruit extract. Values of IC_{50} (µg/mL) demonstrated this difference: Bark extract - 55.12±2.35, Fruit extract - 140.45± 2.90 and BHT 6.97±0.16.

Table 1: Free radical scavenger activity (IC_{50} µg/mL) of extracts of *L. ferrea* and antioxidant standards.

Sample	DPPH	ABTS	$O_2^{\cdot\bullet}$
Libidibia ferrea fruits	7.8 ± 1.2[a]	3.5 ± 0.01[a]	24.6 ± 4.9[a]
L. ferrea bark	7.8 ± 0.9[a]	5.0 ± 0.09[b]	31.7 ± 2.4[a]
Ascorbic acid	2.7 ± 0.3[b]	5.6 ± 0.2[b]	6.9 ± 3.3[b]
Gallic acid	1.1 ± 0.2[c]	0.5 ± 0.02[c]	2.3 ± 1.4[c]

Different letters indicate statistical differences in the same column (ANOVA, $p<0.05$)

Table 2: Cytotoxicity of Bark and Fruits extracts from *L. Ferrea* and doxorubicin, in murine NIH3T3 line cells by Alamar blue assay.

Substance	IC_{50} (µg/mL) [CI95% (µg/mL)]
Bark	62.20 (51.86 to 74.61)
Fruits	64.47 (50.72 to 81.97)
Doxorubicin	0.92 (0.74 to 1.15)

When evaluated in murine NIH3T3 cells, the cytotoxicity (LC_{50}) of bark and fruit extracts of *L. ferrea* was higher than 250 µg/mL after 24 h of treatment, while the LC_{50} of Doxorubicin was 5.782 µg/mL (4.799 to 6.967), as shown in **Table 2**. Both fruit and bark extracts of *L. ferrea* also demonstrated antioxidant activity using a cell-base assay. The bark extract showed better activity than fruit extract and a concentration-dependent curve, and at 30 min, the test showed the greatest performance. At concentration 50 µg/mL, extracts of fruit and bark of *L. ferrea* showed significant antioxidant activity and bark showed more than 60% inhibitions as shown in **Fig 2**. Quercetin at 5

µg/mL was used as the standard and showed around 80% of inhibition.

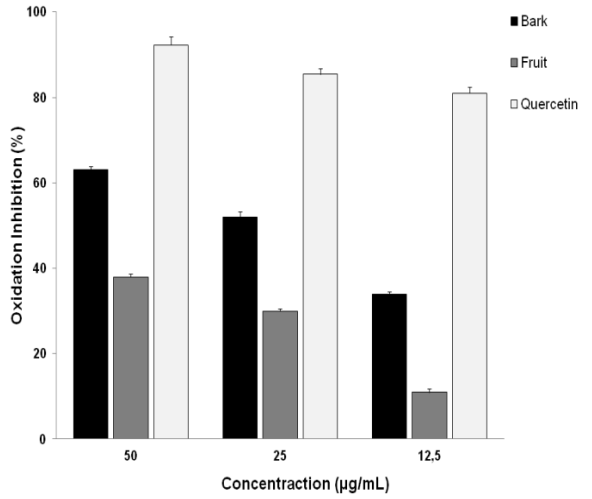

Fig. 2: Effects of extracts from bark and fruit of *L. ferrea* and standard quercetin on oxidation of 2'-7'-dichlorofluorescin in a cell-based assay.

Administration of CCl_4 markedly increased the serum levels of liver enzymes such as serum glutamate oxaloacetate transaminase (GOT), serum glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) compared to the control group. Elevations in the secretion of these enzymes were significantly decreased (P<0.01) by extracts of bark and fruits of *L. ferrea* (100 mg/Kg and 200 mg/Kg) administration by gavage compared to the CCl_4 group is shown in **Table 3**. The treatment with CCl_4 resulted in a significant increase in serum MDA. Treatment of mice with different concentration extracts of bark and fruits of *L. ferrea* resulted in a significant decrease in the serum MDA levels (**Fig. 3**).

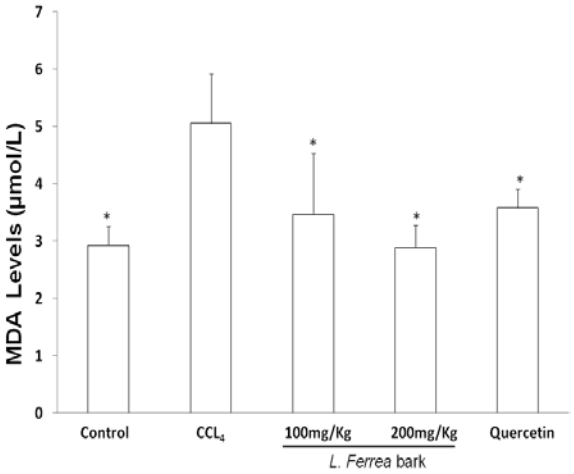

Fig. 3: Effects of extract of *L. ferrea* bark on lipid peroxidation (TBARS) in acute hepatic injury induced by CCl4. (n=5). Values are expressed as mean±standard deviation. * represents statistical significance when compared to the CCl_4 treated group; (ANOVA-Tukey p <0.05)

Table 3: Effect of *L. ferrea* extracts and quercetin on CCl_4 – induced hepatotoxicity in BALB/c mice

Enzymes	Control	CCL_4	*L. ferrea* (100mg/Kg)	*L. ferrea* (200mg/Kg)	Quercetin (50mg/Kg)
GOT (U/L)	45±31[a]	2140±850[b]	1383±45[c]	593±323[d]	343± 303[d]
GPT (U/L)	110± 56[a]	1615± 120[b]	1385± 63[b]	450± 197[c]	475± 233[c]
ALP (U/L)	70±42[a]	105±35[b]	65±71[a]	70±14[a]	80±28,2[a]

Different letters indicate statistical differences on the same line (ANOVA, p<0.05)

DISCUSSION

Polyphenols, particularly flavonoids, are among the most potent plant antioxidants. Polyphenol can form complexes with reactive metals such as iron, zinc and copper reducing their absorption, but excess levels. In addition, polyphenols also function as potent free radical scavengers within the body, where they neutralize free radicals before they can cause cellular damage [21].

The standardized extracts of *L. ferrea* demonstrated excellent antioxidant potential with low IC_{50} in the DPPH, ABTS and O_2^- scavenger assays. The results could be explained by the content of polyphenol compounds, mainly gallic acid and epicatechin, present in the extract as visualized through a chromatography profile **(Fig. 1)** and known to have antioxidant activities. In addition, a similar chromatography profile for the bark extract demonstrates the presence of substances absent in the fruit extract, which could explain the better results for this product.

The DPPH scavenging assay was chosen as the primary test to be performed in an initial screening of extracts due to its relatively low cost and high stability of the reagent [22]. The ABTS radical scavenger test has been extensively described by many different authors and in general is useful for the evaluation of antioxidant activity of substances having lipophilic or hydrophilic properties, including flavonoids, carotenoids and blood plasma [16,23]. In this test, the extraction activity IC_{50} values were lower than values for plant extracts considered antioxidant in the literature (50-100 µg/mL) [24]. Only samples presenting IC_{50} < 10 µg/mL are considered very active antioxidants, comparable to the antioxidant standards quercertin, β-carotene, ascorbic acid, gallic acid and Trolox®[25]. The ability of some extracts to scavenge free radicals in simple tests for the evaluation of antioxidant capacity, such as those based on DPPH and ABTS does not mean that these tests work readily in complex mechanisms such as those present in physiological substrates; thus, the need to verify the antioxidant effect in scavenging specific species such as superoxide anion radical ($O_2^{\bullet-}$). $O_2^{\bullet-}$ is produced constantly in organisms by diverse cellular processes, such as the electron transport chain in mitochondria, in microsomes and through enzymes like xanthine oxidase and NADPH oxidase and can be increased as part of certain pathologies [26].

Our findings could be compared with the antioxidant activity of other species of the genus *Libidibia* that also demonstrated antioxidant potential. Many authors indicate certain terpenoids, steroids and phenolic compounds such as tannins, coumarins and flavonoids have protective effects due to their antioxidant properties [27]. Compared to other plants of different families, the bark and fruit extracts of *Libidibia ferrea* have good antioxidant activity with the DPPH method. The crude hydroalcoholic extract of *Jacaranda decurrens* have 48% inhibition in 1.0 mg/mL [23]. The IC_{50} of bark ethanolic extract of *Calceolaria chelidonioides* is 122.34 µg/mL, which is the best value compared with flowers, roots and leaves [28]. The hydroalcoholic extract of *Arctium lappa* Linne leaves has an IC_{50} of 29 µg/mL[29].

The non-cellular *in vitro* tests discussed above showed that the extracts of bark and fruit of *L. ferrea* exhibit antioxidant activity. A

cellular *in vitro* test was performed, and it was found that both extracts also showed antioxidant activity. The extract of bark showed greatest result than the extracts of fruits of *L. ferrea.* The cellular antioxidant activity assay was performed with dichlorofluorescein and AAPH. Upon entering the cell, releasing peroxyl radicals which AAPH to be incorporated by dichlorofluorescein, increases their fluorescence. Extract antioxidants are capable of capturing the radical preventing an increase in fluorescence [20].

Aiming to verify *in vivo* antioxidant and hepatoprotective activities of *L. ferrea*, a CCL_4 test was performed in mice. Lipid peroxidation is a major parameter that can be included as a marker of oxidative stress. MDA is widely used as a parameter of lipid peroxidation [22]. In this study, increased MDA contents in the CCl_4-treated group suggested that natural antioxidant defense mechanism to scavenge excessive free radicals has been compromised. Treatment of mice with different concentration extracts of bark and fruits (100 and 200 mg/mL) of *L. ferrea* resulted in a significantly diminished level of serum MDA. In a recent similar study [30], they studied the hepatoprotective activity of the total saponins from *Actinidia valvata;* Dunn also found an increase in MDA in CCl_4 groups and a decrease in MDA levels in groups treated with the total saponins from *Actinidia valvata* Dunn. Another study [31] tested attenuation of CCl_4- induced oxidative stress and hepatonephrotoxicity by saudi sidr honey (SSH) in rats and also found results that indicated significant increase in MDA in the group treated with CCl_4, and significantly diminished level of MDA in the group treated with SSH. Quercetin was used as standard in another study [32], which studied the hepatoprotective activity of ethanol extract of *C. sativum* and demonstrated that this search may be due to the antioxidant potential of phenolic compounds.

A number of drugs, toxic industrial chemicals, and viral infections have been reported to cause severe hepatic injuries, which are sometimes difficult to manage by medical therapies. It is important to evaluate plant extracts that can be used for improved treatment of hepatic failure caused by severe oxidative stress [33]. In the present study, administration of CCl_4 to mice caused a significant increase in serum GOT, GPT and ALP as well as MDA, which indicates an extensive disruption of liver function. The tendency of the marker enzymes (GOP, GPT and ALP) to be at near normal levels in the groups of mice treated with extracts from bark and fruits of *L. ferrea* is a manifestation of hepatoprotective activity of this plant. Our findings are in agreement with an earlier study of mice fed extracts from the *Nymphaea candida* flower, which has mainly polyphenol characteristic compounds, such as *L. ferrea* showed [34].

Therefore, extracts of *L. ferrea* appear to protect the liver against CCl4 induced oxidative damage in mice and can be considered a good antioxidant *in vitro* and *in vivo* by the tests performed in this study. These effects might be correlated with their antioxidant and free radical scavenger effects. These assays indicate that this plant extract is a significant source of natural antioxidants, which might help prevent progress of oxidative stress.

CONCLUSION

Both bark and fruit extracts showed high antioxidant activity explained by the presence of polyphenol compounds such as gallic and epicatechin. The administration of bark and fruit extracts from *L. ferrea* prevented liver biochemical alteration induced by CCl_4. This hepatoprotective activity could be attributed to the presence of antioxidant factors such as, phenols and flavonoids, which cause significant lowering of the oxidative threat leading to normal physiological function.

CONFLIT OF INTEREST

The authors declare that there are no conflitof interest.

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