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In silico analysis of active metabolites isolated from *Libidibia ferrea* Martius

Análise *in silico* de metabólitos ativos isolados de *Libidibia ferrea* Martius

Análisis *in silico* de metabolitos activos aislados de *Libidibia ferrea* Martius

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Abstract

The present work carried out *in silico* studies to predict the pharmacokinetic, physicochemical, toxicological and biological activities of isolated metabolites from *Libidibia ferrea* Martius, a plant popularly used for the treatment of inflammations and injuries, such as antidiabetic, antimicrobial, antifungal and antileishmaniasis. Comparisons were made using the following programs: chemsketch, mcule property calculator, preadmet, protox and pass online. The results revealed that only the molecules lupenone and *trans*-farnesol showed intestinal absorption above 90%. The metabolites gallic acid, catechin, methyl-gallate, quercetin and *trans*-farnesol follow the rule of lipinsk in all its preconditioned parameters. With regard to enzyme inhibition, each of the study molecules demonstrated inhibition directed at at least two CYP enzymes. Lupenone was the only molecule that was active against the protozoan leishmania, in addition to being promising against neoplasms and inflammation. Catechin and quercetin demonstrated positive results with regard to antioxidant activity, and most of the substances in the study in question showed mucomembranous protection capacity. No substance demonstrated embryotoxicity or teratogenesis, but all

molecules were found to have a certain level of toxicity in other analysis parameters. In pharmacokinetic, toxicological and biological terms, pauferrol A, B and C molecules were the least promising, while lupenone, catechin and quercetin showed the best results.

Keywords: Medicinal plants; Predictions; Neoplasms; Leishmaniasis.

Resumo

O presente trabalho realizou estudos *in silico* para predição das atividades farmacocinéticas, físico-químicas, toxicológicas e biológicas de metabólitos isolados de *Libidibia ferrea* Martius, planta utilizada popularmente para o tratamento de inflamações e lesões, como antidiabética, antimicrobiana, antifúngica e antileishmaniose. As comparações foram realizadas através dos programas: chemsketch, mcule property calculator, preadmet, protox e pass online. Os resultados revelaram que apenas as moléculas lupenona e o *trans*-farnesol apresentaram absorção intestinal acima de 90%. Os metabólitos ácido gálico, catequina, metil-galato, quercetina e *trans*-farnesol seguem a regra de lipinsk em todos os seus parâmetros pré-condicionados. No que se refere a inibição enzimática, cada uma das moléculas do estudo demonstrou inibição direcionada a pelo menos duas enzimas da CYP. A lupenona foi a única molécula que se apresentou ativa contra o protozoário leishmania, além de ser promissora contra neoplasias e inflamação. A catequina e quercetina demonstraram resultados positivos no que tange a atividade antioxidante, e a maioria das substâncias do estudo em questão apresentaram capacidade de proteção mucomembranosa. Nenhuma substância demonstrou embriotoxicidade ou teratogênese, mas todas as moléculas demonstraram possuir certo nível de toxicidade em outros parâmetros de análise. Em termos farmacocinético, toxicológico e biológico, as moléculas pauferrol A, B e C foram as menos promissoras, enquanto que a lupenona, catequina e quercetina apresentaram os melhores resultados.

Palavras-chave: Plantas medicinais; Predições; Neoplasias; Leishmaniose.

Resumen

El presente trabajo realizó estudios *in silico* para predecir las actividades farmacocinéticas, fisicoquímicas, toxicológicas y biológicas de metabolitos aislados de *Libidibia ferrea* Martius, planta popularmente utilizada para el tratamiento de inflamaciones y lesiones, tales como antidiabético, antimicrobiano, antifúngico y antileishmaniasis. Las comparaciones se realizaron utilizando los siguientes programas: chemsketch, mcule property calculator, preadmet, protox y pass online. Los resultados revelaron que solo las moléculas lupenona y

trans-farnesol mostraron una absorción intestinal superior al 90%. Los metabolitos ácido gálico, catequina, galato de metilo, quercetina y *trans*-farnesol siguen la regla de lipinsk en todos sus parámetros preacondicionados. Con respecto a la inhibición enzimática, cada una de las moléculas de estudio demostró una inhibición dirigida al menos a dos enzimas CYP. La lupenona fue la única molécula activa contra el protozoario leishmania, además de ser prometedora contra las neoplasias y la inflamación. La catequina y la quercetina demostraron resultados positivos con respecto a la actividad antioxidante, y la mayoría de las sustancias del estudio en cuestión mostraron capacidad de protección mucomembranosa. Ninguna sustancia demostró embriotoxicidad o teratogénesis, pero se encontró que todas las moléculas tenían un cierto nivel de toxicidad en otros parámetros de análisis. En términos farmacocinéticos, toxicológicos y biológicos, las moléculas de pauferrol A, B y C fueron las menos prometedoras, mientras que la lupenona, la catequina y la quercetina mostraron los mejores resultados.

Palabras clave: Plantas medicinales; Predicciones; Neoplasias; Leishmaniasis.

1. Introduction

The medicinal plants in Amazon represent the main form of disease treatment for most poor populations, due to cultural influences and the cost of pharmaceutical products (Elisabetsky & Wannamacher, 1993). Among these plants, there is *Libidibia ferrea* (Mart. Ex Tul.), popularly known as Jucá, belonging to the Fabaceae family, widely distributed in the north and northeast regions, and also present in the southeast region. It is a large tree, reaching a height of 15 meters, presenting a smooth bark and hard heartwood and bipinnate leaves with oblong or oval leaflets, with hermaphrodite and yellowish diclamid flowers. This species is used in Amazon mainly to treat inflammation and injuries (Staci & Hiruma-Lima, 2002). Studies show that Jucá is anti-inflammatory (Carvalho, et al., 1996; De Araújo, et al., 2014), antidiabetic (Ueda, et al., 2001) and analgesic (Carvalho, et al., 1996; Lima, et al., 2001). It also has antimicrobial (Carvalho, et al., 1996; Sampaio, et al., 2009), antifungal (Martins, et al., 2014; Rhayanny, et al., 2013), allelopathic (Oliveira, et al., 2012), and chemopreventive activity against cancer (Nakamura, et al., 2002).

Previous analysis highlights the presence of some metabolites in *L. ferrea*, among them: Gallic acid (Figure 1A); *methyl*-gallate (Figure 1B); Catechin (Figure 1C); Quercetin (Figure 1D); di(2-ethylhexyl) phthalate (Figure 1E); Lupenone (Figure 1F); Pauferrol A (Figure 1G); Pauferrol B (Figure 1H); Pauferrol C (Figure 1I); *trans*-Farnesol (Figure 1J).

Studies highlight that the biological activities related to this plant are attributed to these compounds. For example, analyzes demonstrate that catechins (Hosokawa, et al., 2010) and Lupenone (Feng Xu, et al., 2019) have potent anti-inflammatory properties. A study showed the methyl gallate is an expressive antioxidant and induces sustained activation and apoptosis in human brain carcinoma cells (Chaudhuri, et al., 2015). The gallic acid also induces apoptosis in lung cancer cells (Ohno, 1999).

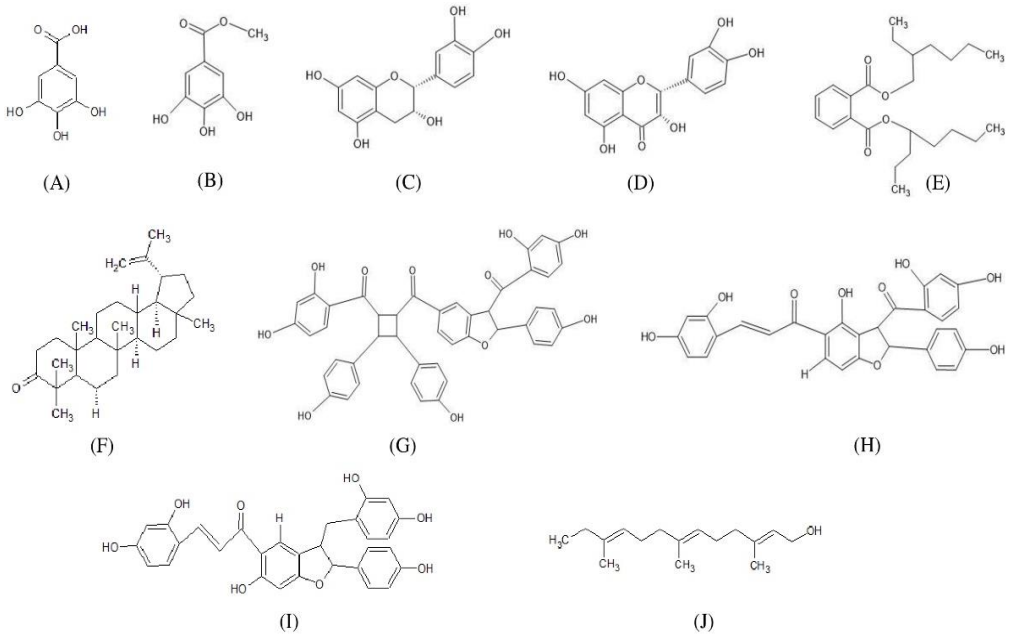
Although investigations prove the importance of these molecules to the plant's biological activities, little is known about their pharmacokinetic and toxicological aspects. This article describes, for the first time, the pharmacokinetic, toxicological, physical-chemical and biological activities obtained by *in silico* studies of molecules isolated from *L. ferrea*.

2. Methodology

2.1 Bibliographic research

This is a quantitative work by comparison (Pereira A.S. et al., 2018) that analyzes the variety physical-chemical, pharmacokinetic, biological activities and toxicological of the designed molecules (Bittencourt, 2017), carried out through a bibliographic review of national and international journals. Ten compounds isolated from different parts of the plant L. ferrea were selected, with different chemical classes, characteristics and varied functions. The bibliographic search was performed on indexed databases of portals and journals (CAPES, Scielo, Google Scholar) without date restrictions.

Figure 1. Substances isolated from *Libidibia ferrea*.



Legend: (A) Gallic acid; (B) methyl-gallate; (C) Catechin; (D) Quercetin; (E) Di (2-ethylhexyl) phthalate; (F) Lupenone; (G) Pauferrol A; (H) Pauferrol B; (I) Pauferrol C; (J) trans-Farnesol. Source: Authors.

2.2 Pharmacokinetics

The ChemSketch program (version 12.1.0.31258) was used to design the chemical structures, which were saved in an appropriate format (.mol) for later analysis in the databases covered in this study. The physicochemical characteristics of the compounds were estimated by Mcule property calculator (Mcule-2019).

The PreADMET (preadmet, 2020) program was used to investigate whether the studied molecules follow the Lipinsk rule (Rule of Five), helpful to detect and validate molecules with "Good absorption by the body". The 5 parameters this rule encompasses are: molecular mass ≤ 500, log P ≤ 5, Polar Surface area ≤ 140 Å2, hydrogen bond acceptors ≤ 10, and hydrogen bond donors ≤ 5. It is important to note, according to the literature, that a polar surface smaller than 90Å2 is the approximate maximum limit for a molecule to cross a blood-brain barrier (Wermuth, 2003).

In pharmacokinetic studies it was possible to evaluate skin permeability (high permeability: < 0.1, and low permeability: > 0.1), permeability in Human Colon Adenocarcinoma Cells (Caco-2 cells) Irvine et al (1999) and Madin-Darby Canine Kidney

(MDCK) Balimane & Chong (2005), with the following parameters: high permeability > 70 nm/sec, medium permeability 4-70 nm/sec and low permeability < 4nm/sec.

The program also evaluated human intestinal absorption (HIA) (Ajay, Bermis, & Murkco, 1999), with HIA weighted 0-20% (low absorption), 20-70% (moderate absorption) and > 70% (high absorption). In addition, the ability of molecules to bind to plasma proteins was verified (high > 90%; moderate to low < 90%)as well as the ability to cross the blood-brain barrier (BBB), using the criteria: freely crosses the BBB > 2.0; crosses moderately 2.0-0.1 and crosses slightly or does not cross < 0.114 (Yazdanian, Glyn, Wright & Hawi, 1998).

For the metabolism predictions, the following criteria were used: if the molecule undergoes phase 1 metabolism, and if it suffers, which enzymes should be targeted. Furthermore, the molecules that induce the enzymes were categorized. It is worth noting that chemical substances that are neither CYP inducing nor inhibiting are ideal substances (Dolabela, et al., 2018).

2.3 Toxicity/Mutagenicity

The PreaDMET program was used to the analyses. The mutagenicity prediction was assessed by the Ames, which cataloged several strains of *Salmonella typhimurium* (TA100_10RLI, TA100_NA, TA1535_10RLI, TA1535_NA). The test assesses whether the substance is capable of causing growth reversion to bacteria in a medium without histidine. The result of the Ames Test was considered positive when there was a reversal of the mutation in one or more bacteria and negative when there was no reversal (Ames, et al., 1975).

Rat and mouse models were used to assess carcinogenic potential. The results of the program were expressed in (+) carcinogenic and (-) non-carcinogenic.

Regarding the assessment of toxicity criteria in marine organisms, the following parameters were considered: toxicity to algae - toxic < 1 mg/L and non-toxic > 1 mg/L; toxicity in *Daphnia sp* - toxic < 0.22 μg/mL and non-toxic > 0.22 μg/mL; toxicity in *Medaka* and *Minnow* fish - very toxic < 1 mg/L, toxic 1-10 mg/L, harmful 10-100 mg/L and non-toxic > 100 mg/L (Costa, Botta & Espindola, 2008; Guilhermino, Diamantino, Silva & Soares, 2008).

In the assessment of oral toxicity, the PROTOX software was used, considering classifications from classes I to VI (fatal if ingested to non-toxic) based on the 50% lethal dose (as can be seen in Table 1). The ProTox-II incorporates molecular similarity, propensity

to fragments, more frequent resources and machine learning (CLUSTER cross-validation based on fragment similarity), based on a total of 33 models for predicting various toxicity endpoints, such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcome paths (Tox21) and toxicity goals (Drwal, et al., 2014).

Table 1. Prediction of oral toxicity in rodents.

CLASSIFICATION	LETHAL DOSE	INTERPRETATION
Class I	$LD_{50} \leq 5$	Fatal if swallowed
Class II	$5 < LD_{50} \leq 50$	Fatal if swallowed
Class III	$50 < LD_{50} \leq 300$	Toxic if swallowed
Class V	$300 < LD_{50} \leq 2000$	Harmful if swallowed
Class V	$2000 < LD_{50} \leq 5000$	May be harmful if swallowed
Class VI	$LD_{50} > 5000$	Non-toxic

Legend: LD - lethal dose. Source: Authors.

2.4 Prediction of Biological Activities

The prediction of biological activity was performed in the PASS software online, where substances were evaluated for similarity with more than 250,000 biologically active molecules, among them, drugs and toxic compounds (Filimonov, et al., 1995). The exclusion criterion is based on Pa (Probability of the substance being active) > 0.7, in parallel to the low number of Pi (Probability of being inactive) Stepanchikova et al (2003).

3. Results

In this study, 10 isolated metabolites of *L. ferrea* were analyzed, among them: the phenolic compounds gallic acid (Figure 1A) and methyl gallate (Figure 1B); the flavonoids catechin (Figure 1C) and quercetin (FIGURE 1D); the di-2-ethylhexyl phthalate (Figure 1E); the triterpene lupenone (Figure 1F); the chalcones Pauferrol A (figure 1G), Pauferrol B (figure 1H) and Pauferrol C (Figure 1I); and the terpene *trans*-Farnesol (Figure 1J).

In the prediction studies about physical-chemical characteristics, the molecular mass of the pauferrol A, B and C exceed 500 g/mol (equivalent to 500 Da), violating one of the rules. The lupenone, pauferrol A and di-2-ethylhexyl phthalate presented Log P > 5. In relation to PSA (Polar Surface Area) the three pauferrol molecules have values greater than 140Å^2. Only pauferrol A exceeded the limit of 10 hydrogen bond acceptors (HBA). The three pauferrol molecules surpassed the limit of 5 hydrogen-bonded donors. Gallic acid, catechin, *methyl*-galate, quercetin and trans-farnesol followed the rule of five in all its parameters (Table 2).

Table 2. Physicochemical prediction of the substances isolated from *L. ferrea*.

Molecules	**Molecular mass (g/Mol)**	**logP**	**PSA***	**HBA***	**HBD***
Phenolic Compounds					
Gallic acid	170.1191	0.5016	97.9900	5	4
methyl-galate	184.1456	0.5900	86.9900	5	3
Flavonoids					
Catechin	290.2671	1.5461	110.3800	6	5
Quercetin	302.2346	1.9880	131.3600	7	5
Phthalates					
Di-2-ethylhexyl	390.5551	6.4330	52.6000	4	0
Triterpenes					
Lupenone	410.6737	7.6571	17.0700	1	0
Chalcones					
Pauferrol A	750.7416	7.6052	202.0500	11	7
Pauferrol B	526.4881	4.9165	164.7500	9	6
Pauferrol C	526.4881	4.9165	164.7500	9	6
Terpenes					
trans-Farnesol	236.3923	4.7880	20.2300	1	1

Legend: Molecular mass - up to 500 g/mol; log P- up to 5; PSA (Polar Surface Area) - up to 140 Å2; HBA (Hydrogen Bond Acceptors) - up to 10; HBD (Number of hydrogen bonding donors) - up to 5. Source: Authors.

The results of the pharmacokinetic predictions suggest that all molecules are well absorbed in a cutaneous form, while the absorption in MDCK cells was low to moderate, with the highest absorption belonging to *trans*-farnesol (58.43 nm/sec). In Caco 2 cells, the molecules lupenone (49.53), di-2-ethylhexylphthalate (46.52) and *trans*-farnesol (33.62) presented the highest absorption rates. Regarding human intestinal absorption, all the target molecules showed moderate to high absorption, with gallic acid (53.69), methyl gallate (69.74), catechin (66.70) and quercetin (36.48) presenting moderate absorption. The molecules highly absorbed were Lupenone (100), *trans*-farnesol (100), pauferrol A, B and C (88.02, 88.09 and 81.49, respectively) (Table 3).

In the plasma protein binding prediction, all molecules (with the exception of phenolic compounds) showed a strong connection with 100% absolute numbers. Among all the substances analyzed, the gallic acid had lowest affinity to plasma proteins (65.38%) (Table 3).

The prediction studies showed that phthalates terpenes and triterpenes cross the blood-brain barrier. The phenolic compounds (gallic acid and methyl gallate) presented low and medium penetration, respectively. The flavonoid catechin, on the other hand, had medium penetration, while the results indicate that quercetin has a low penetration capacity. Finally, the chalcones Pauferrol A and B also have median penetration, while Pauferrol C showed a low index (Table 3).

In the prediction, the lupenone, pauferrol A and B and *trans*-farnesol are metabolized by CYP3A4, while catechin, di-2-ethylhexylphthalate and pauferrol C are weak substrates. In this study, the molecules demonstrated enzymatic inhibition – an important factor for drug discovery - to at least two CYP enzymes, mainly CYP3A4 and CYP2P19, as shown in Table 3.

Table 3. Pharmacokinetic predictions of substances isolated from *L. ferrea*.

Molecules	**Absorption**				**Distribution**		**Metabolism**	**Inhibition**
	Cutaneous	**MDCK (nm/sec)**	**Caco2 (nm/sec)**	**HIA (%)**	**PP (%)**	**BBB**	**Phase 1**	**CYP**
Phenolic Compounds								
Gallic Acid	High	Moderate	Moderate	Moderate	Weak	Low	-	CYP2C9 CYP2C19 CYP3A4
methyl-Gallate	High	Moderate	Moderate	Moderate	Weak	Moderate	-	CYP2C9 CYP3A4
Flavonoids								
Catechin	High	Moderate	Low	Moderate	Large	Moderate	Weak	CYP2C9 CYPC19 CYP3A4
Quercetin	High	Moderate	Low	Moderate	Large	Low	-	CYP2C9 CYPC19 CYP3A4
Phthalates								
Di-2-ethylhexylphthalate	High	Low	Low	High	Large	High	Weak	CYP2C9 CYPC19 CYP3A4
Triterpenes								
Lupenone	High	Low	Moderate	High	Large	High	CYP3A4	CYP2C9 CYP3A4
Chalcones								
Pauferrol A	High	Low	Moderate	High	Large	Moderate	CYP3A4	CYP2C9 CYPC19 CYP3A4

Pauferrol B	High	Low	Moderate	High	Large	Moderate	CYP3A4	CYP2C9 CYPC19 CYP3A4
Pauferrol C	High	Low	Moderate	High	Large	Low	Weak	CYP2C9 CYPC19 CYP3A4
Terpenes								
trans-Farnesol	High	Moderate	Moderate	High	Large	High	CYP3A4	CYP2C9 CYPC19 CYP3A4

Legend: MDCK- high absorption > 70nm/sec; Caco 2- high absorption > 70 nm/sec; HIA (Human Intestinal Absorption) - 0-20% low, 20-70% moderate and > 70% high; PP (Plasma Proteins) - high absorption > 90%; moderate to low absorption < 90%; BBB (blood-brain barrier) - freely crosses > 2.0, moderate from 2.0-0.1 and crosses in a reduced way < 0.1. Source: Authors.

The results obtained by PASS also helped in the identification of molecules and their interactions with CYP enzymes. The data are shown in Table 4, in which 17 interactions were cataloged with a Pa value above 0.7 (criterion adopted above). The molecules that "inhibit" and "induce" the same CYP were eliminated, showing the program limitation. In this context, quercetin presented more expressive numbers, interacting with a greater number of CYPs.

Table 4. Interactions with CYP enzymes in the predicting PASS.

Molecules	Interactions with CYP enzymes
Phenolic Compounds	
Gallic Acid	CYP2J (S), CYP2J2 (S)
methyl-Gallate	CYP2H (S)
Flavonoids	
Catechin	CYP1A (S), CYP3A (I), CYP2A4 (S)
Quercetin	CYP2C12 (S), CYP1A2 (I), CYP19 (I), CYP1B1(I), CYP2C (S)
Triterpenes	
Lupenone	CYP2J (S)
Terpenes	
trans-Farnesol	CYP2J(S), CYP2E1 (I)

Legend: S - substrate; I – inhibition. Source: Authors.

All structures subjected to Algae toxicity predictions were considered toxic, with the exception of Pauferrol A. In the toxicity assessment on crustaceans Daphnia sp., Only the *methyl* Galate and *trans*-Farnesol showed negative results to possible toxic events. Tests on Medaka fish revealed that all molecules have a certain ability to cause some toxic event. The catechin, di-2-ethylhexylphthalate, lupenone and Pauferrol A, were very toxic (<1mg/L). In the evaluation of cytotoxicity, the molecules C, G and I seem to inhibit the hERG Channel. (Table 5).

The substances A, B (phenolic compounds), C, D (flavonoids) and F were positive for possible mutagenic events. The study of carcinogenicity in rats demonstrated that the phenolic compounds, quercetin, di-2-ethylhexylphthalate and lupenone are positive to the probability of causing a carcinogenic event. The same study carried out in mice reveals that di-2-ethylhexylphthalate, pauferrol B and C, and *trans*-Farnesol can be toxic but only the compound di-2-ethylhexylphthalate would be capable of causing toxicity in the two animals. The compounds did not show embryotoxic or teratogenic characteristics (Table 5).

Table 5. Toxic events of isolated substances from *L. ferrea*.

Parameters	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Toxicity										
Algae	+	+	+	+	+	+	-	+	+	+
Daphinia sp.	+	-	+	+	+	+	+	+	+	-
Medaka Fish	+	+	++	+	++	++	++	+	+	+
Minnow Fish	+	+	+	+	-	-	-	-	-	+
Cytotoxicity										
Cytotoxic *	-	-	+	±	-	-	+	±	+	-
Mutagenicity										
Mutagenic	+	+	+	+	-	+	-	-	-	-
Carcinogenicity										
Rats	+	+	-	+	+	+	-	-	-	-
Mice	-	-	-	-	+	-	-	+	+	+
Embryotoxicity and Teratogenesis										
Embryotoxic and teratogenic	-	-	-	-	-	-	-	-	-	-

Legend: A- Gallic acid; B-*methyl* gallate; C- Catechin; D- Quercetin; E-Di-2-ethylhexyl phthalate; F-Lupenone; G- Pauferrol A; H- Pauferrol B; I- Pauferrol C; J- trans-Farnesol. (+) = Toxic; (++) = Very toxic; (-) Non-toxic. * HERG channel: (+) moderate risk; (++) high risk; (-) low risk. Source: Authors.

The prediction of acute oral toxicity suggests the pauferrol B and C can be fatal if swallowed. Pauferrol A can be harmful if ingested, as well as gallic acid, lupenone and *trans*-farnesol. Methyl gallate and di-2-ethylhexyl phthalate can be harmful if swallowed, while quercetin proved to be toxic if ingested.

Lupenone and Pauferrol B and C presented immunotoxic effects. Gallic acid and di-2-ethylhexyl phthalate were characterized as potential carcinogenic molecules. The quercetin had the greatest possible toxic effects and may be carcinogenic and mutagenic (Table 6). The

catechin was the only substance which showed a promising result in acute oral toxicity prediction, non-toxic if ingested and without adverse effects (Table 6).

The catechin binds to an interaction target (PGH1) although it is non-toxic and has no toxic effect, perhaps due to the fact that it was active in the Mitochondrial Membrane Potential (MMP) in which the stress response pathways take place. The pauferrol A also presented targets of interaction (PGH1, RE and RhA), however it did not have results that indicate adverse effects, maybe due to manifested effects on MMP and P53 phosphoprotein (Fp53) in the cellular response to stress (Table 6).

Table 6. Oral toxicity prediction of molecules isolated from *L. ferrea.*

Molecules	**DL $_{50}$ (mg/Kg)**	**Toxic Effect**	**Potential targets for interaction**	**Cellular Stress Response**
Phenolic Compounds				
Gallic Acid	2000 (harmful if swallowed)	carcinogenic	AR	-
methyl-Gallate	1700 (harmful if swallowed)	-	AR and PGH1	-
Flavonoids				
Catechin	10000 (non-toxic)	-	PGH1	MMP
Quercetin	159 (toxic if swallowed)	Carcinogenic and Mutagenic	AR, AO, PGH1, AhR e ER	-
Phthalates				
Di-2-ethylhexylphthalate	1340 (harmful if swallowed)	Carcinogenic	AO e PGH1	-
Triterpenes				
Lupenona	5000 (harmful if swallowed)	Immunotoxic	AO	-
Chalcones				
Pauferrol A	3000 (harmful if swallowed)	-	PGH1, ER and AhR	MMP e Fp53
Pauferrol B	4 (fatal if swallowed)	Immunotoxic	ER e AhR	

Pauferrol C	10 (fatal if swallowed)	Immunotoxic	ER e AhR	
Terpenes				
trans-farnesol	5000 (harmful if swallowed)	-	AO	

Legend: LD_{50}- 50% lethal dose; AR- androgen receptor; AO - amino oxidase A; PGH1- Prostaglandin G/H synthase 1; AhR - Aryl Hydrocarbon Receptor; ER- Alpha Estrogen Receptor. MMP - Mitochondrial Membrane Potential; Fp53- P53 phosphoprotein. Source: Authors.

The metabolites were not active against bacteria and fungus. Furthermore, only trans-farnesol showed antiviral activity, more specifically for the Rinovirus group. Lupenone was the only molecule active against protozoa, specifically leishmania (Table 7).

The metabolites D, F, and H presented anti-inflammatory activity. Other molecules seem to be anti-inflammatory through different routes. According to the *in silico* results, quercetin is able to inhibit the expression of the nitric oxide synthase type 2 (NOS2). Catechin was suitable for inhibiting histamine release.

Through these predictive studies, we observed that only the molecules C and F presented antineoplastic activity, the possible mechanism of action may be the induction of apoptosis by stimulating the production of caspases 3 and 8. The quercetin is able to inhibit the expression of MMP-9 and JAK-2, in addition to inducing apoptosis and potentiating the expression of TP53 (Table 7).

We observed that molecules A, C, D and H are capable of potentiating the expression of Apolipoprotein A-1 (APOA1). In addition, the molecules C and D have antioxidant activities, and quercetin inhibits the synthesis of nitric oxide (NO) and was shown to be positive as a scavenger of free radicals (Table 7). The gallic acid and methyl-gallate are able to inhibit factor D and the substances A, C, D, F and H are able to develop mucomembranous protection (Table 7).

Table 7. Biological activities prediction of substances isolated from *L. ferrea*.

Activity	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Cancer										
Antineoplastics	-	-	+	-	-	+	-	-	-	-
Apoptosis induction	-	-	-	+	-	+	-	-	-	-
Stimulation of caspase 3	-	-	-	-	-	+	-	-	-	-
Stimulation of caspase 8	-	-	-	-	-	+	-	-	-	-
MMP-9 expression inhibitor	-	-	+	+	-	-	-	-	-	-
TP53 expression enhancer	+	-	+	+	-	-	-	-	-	-
Inhibition of JAK 2 expression	+	+	+	+	-	-	-	-	-	-
Microorganisms and parasites										
Antibacterial	-	-	-	-	-	-	-	-	-	-
Antifungal	-	-	-	-	-	-	-	-	-	-
Antiviral	-	-	-	-	-	-	-	-	-	+
Antiprotozoal	-	-	-	-	-	+	-	-	-	-
Anti-inflammatory										
Anti-inflammatory	-	-	-	+	-	+	-	+	-	-
Anti-eczema	+	-	-	-	-	+	-	-	-	+
NOS2 expression inhibitor	-	-	-	+	-	-	-	-	-	-
Histamine release inhibitor	-	-	+	-	-	-	-	-	-	-
Complement factor D inhibitor	+	+	-	-	-	-	-	-	-	-
Anaphylotoxin receptor antagonist	+	+	-	+	-	-	-	-	-	-

Metabolic changes and effect on oxidative stress										
Hypolipemic	-	-	-	-	-	-	-	-	-	-
Cholesterol synthesis inhibition	-	-	-	-	+	-	-	-	-	-
Enhancer of APOA1 expression	+	-	+	+	-	-	-	+	-	-
Increased insulin release	-	+	-	-	-	-	-	-	-	-
Antioxidant	-	-	+	+	-	-	-	-	-	-
Inhibition of NO synthesis	-	-	-	+	-	-	-	+	-	-
Free radical scavenger	-	-	+	+	-	-	-	-	-	-
Mucomembranous Protection										
Mucomembranous Protection	+	-	+	+	-	-	-	+	-	+

Legend: A- Gallic acid; B-*methyl* gallate; C- Catechin; D- Quercetin; E-Di-2-ethylhexyl phthalate; F-Lupenone; G- Pauferrol A; H- Pauferrol B; I- Pauferrol C; J- *trans*-farnesol. (+) toxic; (-) non-toxic. Source: Authors.

Regarding adverse events, the most promising substances, Catechin, Lupenone and Quercetin, have few adverse events in relation to Gallic Acid and methyl-Gallate. Quercetin had relatively marked nephrotoxicity. Catechin, on the other hand, has events linked to behavior, serving as a warning to various tests, requiring further studies in neuroscience (Table 8).

Table 8. Adverse events of substances isolated from *L. ferrea*.

Activity	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Central Nervous system										
Behavioral disorder	-	-	+	-	-	-	-	-	-	-
Metabolic alterations										
Hypercholesterolemia	+	+	-	-	-	+	-	-	-	-
Renal system										
Nephrotoxicity	-	-	+	+	-	-	-	-	+	-
Others										
Muscle weakness	+	+	-	-	-	-	-	-	-	-
Ototoxicity	-	-	-	-	-	-	-	-	-	+
Irritation	+	-	-	-	+	+	-	-	-	+

Legend: A- Gallic acid; B-*methyl* gallate; C- Catechin; D- Quercetin; E-Di-2-ethylhexyl phthalate; F-Lupenone; G-Pauferrol A; H- Pauferrol B; I- Pauferrol C; J- *trans*-farnesol. (+) toxic; (-) non-toxic. Source: Authors.

4. Discussion

Through the results, we noticed that phenolic compounds (Gallic acid and methyl Gallate), flavonoids (Catechin and Quercetin) and *trans*-Farnesol would be the ideal molecules for good oral absorption, since these compounds met all requirements in Lipinski's rule. However, some therapeutic classes as antifungals, antibiotics and cardiotonics do not follow the rule of five, highlighting the possibility of do not abandon molecules even though they are not consistent with the physical-chemical standard (Lipinsk, 2004).

Pharmacokinetic studies revealed that gallic acid, methyl-galate and *trans*-farnesol showed moderate absorption in MCDK and Caco2 cells, and this action can occur due to their low molecular weight and low PSA values. In the item Human Intestinal Absorption (AIH), Lupenone, *trans*-Farnesol, Pauferrol A, B and C stand out, probably due to the LogP value of such compounds, especially Lupenone.

The results regarding phase 1 metabolism suggest Catechin, di-2-ethylhexylphthalate and Pauferrol C are weak substrates. This situation may occur due to the liposolubility of such compounds, which can generate a longer half-life in the organism and take the enzymes (mainly liver) for transforming them into more water-soluble compounds and directing them to elimination.

Regarding the theme of enzymatic inhibition, all the molecules inhibit at least two CYP enzymes, showing the possibility of interference and interactions with different classes of drugs, which may generate toxic effects to the organism (Goodman, 2006).

In this context, it is necessary to resort to physico-chemical information in order to categorize them into different levels of inhibition. For example, there are compounds with less liposolubility, which have weaker levels of inhibition due to this characteristic. Greater inhibition of the enzyme complex is also related to greater numbers of hydrogen bond acceptors (Lin & Lin, 1997). Consequently, molecules such as *methyl*-Gallate, Gallic acid and Quercetin, although they are inhibitors, have low liposolubility and thus, less force by reversible binding at the active sites of enzymes compared to the other targets of the study. Molecules with high values of hydrogen acceptors, on the other hand, should inhibit enzymes more voraciously and are possible drugs candidates, such as Pauferrol A, B and C (Dolabela, et al., 2018). In summary, most of the metabolites showed acceptable values of absorption, binding to plasma proteins and enzymatic induction.

In the toxicity evaluation (Table 5), the PreADMET evaluation in algae showed that all substances were considered toxic, with the exception of Pauferrol A. On *Daphnia sp* crustaceans, only methyl-Gallate and *trans*-Farnesol were not toxic. In general, the toxic events of the isolated substances were quite different between the molecules and their classes, demonstrating the wide variety and complexity of the major components.

Acute oral toxicity studies revealed that Di-2-ethylhexyl phthalate can be a carcinogenic agent, which can be explained by the fact that it is considered a polluting and toxic agent to humans, especially to the reproductive system (Ji-Young, Yang-Hoon & Jiho, 2006). In view of the results, it is clear that the molecule with the lowest toxicity index is *trans*-Farnesol (Table 9).

The prediction of biological activities demonstrated the metabolites did not have antibacterial or antifungal activity, which contradicts some previously studies. For example, there is a report of the antimicrobial activity of catechins, which comes from its effect on proteins of the pathogen cells: by binding to such cells, they cause their precipitation and, consequently, damage the cell wall and interfere in its biosynthesis (Ji-Young, Yang-Hoon &

Jiho, 2006). It has also been reported that *trans*-Farnesol has the potential to affect biofilms of *Steptococcus mutans* through changes in the proton-driving force, possibly due to the interaction of lipophilic domains with the bacterial membrane[2].

In addition, the investigation of the possible action of Lupenone against leishmaniasis may explain the inhibitory activity the extracts obtained from *L. ferrea* have on *in vitro* growth of L. (L.) amazonensis and L. (V.) guyanensis (Shimamura, Zhao & Hu, 2007; Jeon, et al., 2011).

The anti-inflammatory activity was investigated in some molecules (Gallic Acid, Catechin, Lupenone, methyl-Galate, Quercetin, Pauferrol B and *trans*-Farnesol). The *in silico* results referring to catechins are in line with the *in vitro* results, since the literature confirms the anti-inflammatory potential of catechins, since they showed inhibitory properties against various proteins of human intestinal epithelial cells involved in inflammation, including the nuclear factor kappa B (Falcão, 2010).

There is a congruence between *in silico* and *in vivo* studies related to Lupenone, since its anti-inflammatory activity was verified in animal experiments (mice and rats), where the molecule had a significant inhibitory effect on acute and subacute inflammation and inhibited pancreatic inflammation in diabetic rats (Yang, et al., 2001). The significant antidiabetic activity of Lupenone (Feng, et al., 2019; Na & Kim, 2009) may explain why *L. ferrea* is used in folk medicine against diabetes (Ji-Young, Yang-Hoon & Jiho, 2006). It is suggested this ability is also linked to the possible anti-inflammatory potential of the plant.

The Gallic Acid, Catechin, Quercetin, Pauferrol B and trans-Farnesol were positive to mucomembranous protection. The anti-inflammatory potential and the ability to produce mucomembranous protection may be associated with the anti-ulcer property of *L. ferrea*, since the infusion of this plant fruits is recommended to treat oral wounds and control gastric problems (Wu, ., 2017). It is known that mucus production is involved with the defense of surfaces in a living being, and there is an emphasis on the stomach mucosa, since mucus in the stomach is a protection against gastric acid (Dolabela, et al., 2018). Mucomembranous protection can also be associated with the usage of this plant as an expectorant in folk medicine to clean bronchi and bronchioles in respiratory infections.

Lupenone has proved to be the most promising substance against cancer, which is in line with *in vitro* studies, since lupenone has anticancer activity against MCF-7 breast cancer cells in neutral red test (Cavalcante, 2017). The results also showed that Lupenone has the ability to act on specific types of cancer, such as melanone, colorectal cancer, lung cancer, breast cancer, ovarian cancer and leukemia.

Gallic acid, catechin, Pauferrol B and quercetin presented themselves as substances capable of enhancing the expression of APOA1, which is the main structural and functional protein component of high-density lipoprotein (HDL), or "good cholesterol" in plasma. The POA1 defines the size and shape of HDL, solubilizing its lipid component and helping in the reverse transport of cholesterol (Phillips, et al., 1997). From this perspective, it is suggested that such compounds are able to act in cholesterol control, thus decreasing the risk of cardiovascular diseases, such as infarction and atherosclerosis.

Catechin and quercetin showed positive results as antioxidant. Antioxidants, in particular, produce an effective protective action against oxidative damage often involved in the etiology and progression of many human diseases, including cancer (Esterbauer & Cheeseman, 1990). The ability to eliminate free radicals are probably related to the chemopreventive action of the plant, and the molecules that act on the antioxidant activity may act in synergy with the metabolites of antineoplastic functioning.

Table 9. Association of information about the metabolites isolated from *L. ferrea*.

	Pharmacokinetics			**Toxicity**						
Metabolites	**Lipinsk**	**PPB**	**CYP Inhibition**	**Carci**	**Muta**	**Cyto**	**Embryo**	**Anti-cancer**	**Antiparasitic**	**Anti-inflam**
Gallic acid	Yes	No	5	+	+	-	-	-	-	-
methyl gallate	Yes	No	3	+	+	-	-	-	-	-
Catechin	Yes	Yes	5	-	+	+	-	+	-	-
Quercetin	Yes	Yes	8	+	+	±	-	-	+	+
Di-2-ethylhexyl phthalate	No	Yes	3	+	-	-	-	-	-	-
Lupenone	No	Yes	3	+	+	-	-	+	-	+
Pauferrol A	No	Yes	3	-	-	+	-	-	-	-
Pauferrol B	No	Yes	3	-	-	±	-	-	-	+
Pauferrol C	No	Yes	3	-	-	+	-	-	-	-
Trans-Farnesol	Yes	Yes	5	-	-	-	-	-	+	-

Legend: PPB- high plasma protein binding > 90%; CYP inhibition - number of isoforms inhibited; Carci = Carcinogenicity; Muta = Mutagenicity; Cyto = Cytotoxicity; Embryo = Embryotoxicity; Anticancer = Anticancerigen; Antiparasitic = Antiparasitic; Anti-inflam = Anti-inflammatory. Source: Authors.

5. Conclusion

In the prediction of biological activities, only Lupenone was active against protozoa, more specifically against Leishmania spp. The Gallic acid, Catechin, Lupenone *methyl*-Gallate, Quercetin, Pauferrol B and *trans*-Farnesol presented possible anti-inflammatory activity, especially Lupenone. This last molecule also can be promising as an antineoplastic, since it had the greatest amount of positive results regarding the ability to act on different types of cancer. The metabolites Gallic Acid, Catechin, Quercetin, Pauferrol B and *trans*-Farnesol, showed promising results related to mucomembranous protection, ratifying the popular use of the plant. Such results require pre-clinical and clinical studies to prove them, and to prove the potential activities found in this study, it would be extremely important that *in vitro* research be initiated with the ethanol extract of *L. ferrea*.

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