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Annotation of Alkaloids of *Fusaea longifolia* and Evaluation of Anti-*Plasmodium* Activity *in vitro* and *in silico*

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Isoquinoline alkaloids, especially from the Annonaceae family, have shown biological potential against parasites. Thus, this study aimed to evaluate the potential of the alkaloid fractions of the plant *Fusaea longifolia* against *Plasmodium falciparum* and annotate the compounds present in these samples. The tentative characterization of the alkaloids from the leaves and branches of *F. longifolia* was performed using liquid chromatography coupled to mass spectrometry (LC-MS/MS) and molecular networks. Through manual interpretation of the MS/MS spectra, 18 alkaloids were dereplicated from *F. longifolia*, 17 of which were reported for the first time in this species. An unpublished putative glycosylated alkaloid was annotated by interpreting the fragmentation data profile. Regarding biological activity, the fractions studied showed high activity against *P. falciparum* with half-maximal inhibitory concentration (IC_{50}) of 2.42 and 1.60 μg mL^{-1} for branches and from the leaves, respectively, both similar to the reference standard quinine (IC_{50} of 1.24 μg mL^{-1}). The structures of the 17 alkaloids were subjected to *in silico* analysis using molecular docking against four enzymes related to anti-*Plasmodium* activity (wild type (dm-P*f*DHFR) and mutant type (qm-P*f*DHFR), dihydroorotate dehydrogenase (P*f*DHODH) and purine nucleoside phosphorylase (P*f*PNP)). Molecular docking revealed strong interactions, especially between oxoxylopine **17** and hydroxycassythicine *N*-oxide **10**, which may be potential new sources against *P. falciparum*.

Keywords: *Fusaea longifolia*, *Plasmodium falciparum*, LC-MS/MS, molecular networking, molecular docking

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Introduction

Plants of the Annonaceae family from the Amazon have been the target of studies aimed at elucidating their chemical composition in order to use their molecules in the fight against diseases.[1] Among the molecules that have been discovered, alkaloids with an isoquinoline skeleton stand out since several biological properties are attributed to them, such as anticancer,[2] anti-inflammatory,[3] and antimalarial properties.[4] On the other hand, due to the large number of species of the Annonaceae family, many species are still unstudied or understudied from the chemical and pharmacological points of view. One of these is the species *Fusaea longifolia* (Aubl.) Saff, which is popularly known as "*envira*" in Brazil and is distributed throughout the Brazilian Amazon.[5] To date, only five alkaloids have been reported for *Fusaea longifolia* (Aubl.) Saff, one tetrahydroprotoberberine, one aporphine and three oxoaporphines.[6,7] In addition to the fixed components, the composition of the essential oil from the aerial parts, which has trypanocidal activity, has been reported, in which sesquiterpenes were the majority, especially β-selinene, *cis*-β-guaiene and (*Z*)-α-bisabolene.[8]

Malaria remains one of the most serious and potentially fatal infectious diseases in many tropical and subtropical countries, and is caused by parasites such as *Plasmodium falciparum*, which are transmitted to people through the bite of *Anopheles* mosquitoes (infected females).[9] *P. falciparum* is considered the most dangerous species, as this protozoan is the main etiological agent involved in cases of severe malaria and, consequently, is the main cause of deaths from the disease.[10] In 2021, there were 247 million new infections and 619,000 deaths worldwide. Africa accounted for 95% of all deaths and almost all cases of malaria in this region are due to infection by *P. falciparum*.[10] In Brazil, malaria is a major public health problem. In 2019, 157.454 cases and 1.912 hospitalizations were reported, with 83% of cases occurring in the northern region.[11] In addition, *P. falciparum* has demonstrated resistance to existing antimalarial drugs. This generates greater suffering for those infected and is a current challenge for researchers since there is now a need to identify new antimalarial drugs.[12] In this sense, the alkaloids reported in the family Annonaceae have shown promise against *Plasmodium* spp.[13]

Because numerous proteins are essential in various metabolic processes in *Plasmodium falciparum* parasites, they are used as molecular targets for the evaluation of potential antimalarial agents. Among the main molecular targets, the dihydrofolate reductase proteins of the wild type (dm-P*f*DHFR) and mutant type (qm-P*f*DHFR), dihydroorotate dehydrogenase (P*f*DHODH) and purine nucleoside phosphorylase (P*f*PNP) can be highlighted.[14-16] Inhibition of these enzymes disrupts deoxyribonucleic acid (DNA) synthesis or the production of biomolecules that are necessary for parasitic multiplication, which represents a promising route for the development of new antimalarial agents.[17]

Given this scenario, the investigation of alkaloids that are potentially useful against the target enzymes in malaria continues to attract the interest of a number of research groups, which employ various different approaches. Among these, approaches based on mass spectrometry (MS) aiming at the rapid characterization of known molecules in extracts rich in alkaloids have been routinely applied.[18] More recently, the ability to process MS data in open-access platforms, such as Global Natural Products Social Molecular Networking (GNPS), have aided in data interpretation and screening for unknown alkaloids.[19]

Thus, the objective of this study was the chemical characterization of the alkaloid fractions of leaves and branches of *F. longifolia* by means of high-performance liquid chromatography coupled to mass spectrometry (LC-MS/MS) with the aid of molecular networks, as well as the *in vitro* evaluation of the alkaloid fractions against *P. falciparum* and cytotoxic evaluation against healthy human lung fibroblast lineage (MRC-5) cell line. In addition, the dereplicated structures in the alkaloid fractions were evaluated *in silico* against four different proteins that are essential for parasitic multiplication of *P. falciparum*.

Experimental

Plant material

The leaves and branches of *F. longifolia* (Aubl.) Saff. were collected in February 2017 at the Museu da Amazônia (MUSA), Manaus, Amazonas, Brazil (3°00'11.4'S; 59°56'22.8'W), and were identified in the Herbarium of the Instituto Nacional de Pesquisa da Amazônia (INPA), where a voucher (number No. 281627) was deposited. After collection, the botanical material was dried at room temperature (ca. 25 °C) for 72 h and then subjected to pulverization in a knife mill, which yielded 314.5 and 405.7 g of powdered leaves and branches, respectively. Subsequently, the pulverized material was subjected to extraction via maceration with *n*-hexane from Dinâmica (Indaiatuba, SP, Brazil) for 48 h (3 × 1 L) and then with methanol (MeOH) from Dinâmica (Indaiatuba, SP, Brazil) for 48 h (3 × 1 L), yielding 38.21 and 29.60 g of leaf and twig extract, respectively. Immediately after the macerations, aliquots of 10 g of each methanolic extract

were subjected to the acid-base extraction procedure according to a previously described methodology,[20] which yielded 217.4 and 161.7 mg of alkaloid fractions of leaves and branches, respectively. This research was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under the code AFF9974.

LC-MS/MS analysis

The analyses were carried out on an ultra-high performance liquid chromatography (UHPLC) system (Shimadzu Nexera X2, Shimadzu, Kyoto, Japan) coupled to a quadrupole time-of-flight mass spectrometer (MicroTOF-QII; Bruker Daltonics, MA, USA) with an electrospray source, using a concentration of 1 mg mL^{-1} of the alkaloid fractions. The separation of 5 µL samples was performed using a Luna C18 column (150 × 4.6 mm, 2.1 µm) (Phenomenex, USA) at 50 °C, which were eluted at a flow rate of 0.35 mL min^{-1}, isocratically, using (15:85) MeCN/Milli-Q H_2O (both acidified with 20 mM formic acid) from 0 to 2 min and sequential linear gradient up to 95% MeCN for 12 min. The gradient was held for 5 min, followed by a 4 min equilibration at 15% B prior to the next injection. The electrospray ionization conditions (positive mode) were set as follows: capillary potential of 4500 V, temperature of drying nitrogen gas 200 ºC at a flow rate of 9 mL min^{-1}, nebulizer pressure of 4 bar. Mass spectra were acquired using electrospray ionization in the positive mode over an *m/z* range from 50 to 1200. The QTOF instrument was operated in scan and Auto MS/MS mode, and MS/MS experiments were performed on the five most intense ions from each MS survey scan. Accurate mass data were processed using Data Analysis 4.2 software (Bruker Daltonics, Bremen, Germany).[21]

Construction of molecular networks and annotation

For the comparison of the metabolite profiles of the leaves and branches, as well as alkaloid annotation, the product ion spectra resulting from the LC-MS/MS analysis of *F. longifolia* alkaloid fractions were analyzed and organized in molecular networks using the GNPS platform.[22] Tandem mass spectra data was converted to the format .mzXML with MS-convert software 3.0.2113246[23] and then loaded onto the GNPS platform. Parameters for molecular network generation were defined as follows: mass of precursor ions with tolerance of 0.05 Da, product ion tolerance of 0.5 Da, ions below 10 counts were removed from MS/MS spectra. Molecular networks were generated using a cosine score of 0.6. Data were visualized using the software Cytoscape 3.7.0.[24] Annotation of isoquinoline alkaloids present in the samples was performed by manual interpretation of MS/MS spectra compared to the IQAMDB (IsoQuinoline and Annonaceous Metabolites Data Base) database.[20] The molecular networks generated in this study are available for consultation.[25]

In vitro anti-*Plasmodium* activity

Antimalarial activity against *P. falciparum* (FRC3 strain) was tested via flow cytometry using the traditional technique of candle burning in a desiccator providing an atmosphere rich in carbon dioxide and poor in oxygen.[26] The strain was maintained in incomplete Roswell Park Memorial Institute Medium (RPMI) with 10% human serum and fed with normal human erythrocytes A+ at 37 °C. The antiparasitic test of the alkaloid fractions was performed in triplicate with 2% hematocrit and 3 to 5% parasitemia using quinine as the reference drug. The stock solutions were prepared in dimethyl sulfoxide (DMSO) from Nuclear (Diadema, SP, Brazil) (0.02-0.05% final concentration) and serially diluted in the same culture medium (concentrations from 100 to 0.01 µg mL^{-1} in five dilutions). The reading was performed after 72 h by quantifying the percentage of parasitemia in a flow cytometer (FAC-SCAN; Becton Dickinson, NJ, USA) with the use of ethidium bromide dye (Amresco, Solon, OH, USA). The inhibitory concentration at 50% (IC_{50}) was determined from the dose-response curve of *F. longifolia* fractions *vs.* parasitized red blood cells. The percentage of inhibition of parasite growth was determined using the formula of Lopes *et al*.[27] The concentration responsible for 50% inhibition of total parasitemia (IC_{50}) was calculated using GraphPad Prism 8[28] software based on a logarithmic plot of dose *versus* inhibition (expressed as a percentage relative to the control) using nonlinear regression analysis.

Cytotoxic activity

The cytotoxicity of the fractions was evaluated on the proliferation of non-cancerous cell lines MRC5 (human lung fibroblast), which were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, UEA). Cell viability was verified using the Alamar Blue assay as previously described[29] with minor modifications.[30] Cells were cultured as recommended by ATCC guidelines, and a Mycoplasma Staining kit (Sigma-Aldrich, São Paulo, Brazil) was used to confirm that the cells were free of contamination. Doxorubicin (purity ≥ 95%, doxorubicin hydrochloride, IMA S.A.I.C. Laboratory, Buenos Aires, Argentina) was used as the positive control. The values

of IC_{50} with 95% confidence intervals were obtained via nonlinear regression using GraphPad Prism (Intuitive Software for Science).[28]

Molecular docking

The molecular docking assay was performed according to the approach indicated by Santos *et al*.[31] First, the 3D structures of the alkaloids were generated and verified in relation to the protonated state at pH 7.4 and the tautomers via Marvin Sketch software.[32] Then, the structures were optimized for the conformations with lower energy using the semi-empirical method PM7 using MOPAC2016 software.[33] The refined structures were converted to PDBQT files via Autodock tools.[34] The crystal structures of the protein targets were obtained from the Protein Data Bank (PDB). The selected structures of *P. falciparum* were dm-P*f*DHFR (PDB ID: 1J3J), P*f*DHODH (PDB ID: 1TV5), qm-P*f*DHFR (PDB ID: 1J3K) and P*f*PNP (PDB ID: 5ZNC). All four proteins are complexed with the reference drugs pyrimetamine, teriflunomide, WR99210 and quinine, respectively. Finally, molecular docking was performed using Autodock Vina[35] and the results were visualized in Discovery Studio.[36] The validation of the docking protocol was done by means of redocking, seeking conditions of root-mean-square deviation (RMSD) < 2 Å.[34,35]

Results and Discussion

LC-MS analysis and molecular networking

In order to characterize the alkaloids in the leaves and branches of *F. longifolia*, we performed LC-MS/MS analysis, from which 18 different chemical species were annotated (15 in the leaves and 13 in the branches) (Figure 1). All the compounds that were annotated are from the class of isoquinoline alkaloids. In particular, molecules with tetrahydroprotoberberine (two), benzylisoquinoline (seven), aporphine (five) and oxoaporphine (three) skeletons were observed. These alkaloids were identified based on the observation of *m/z* pairs, referring to protonated structures containing only one nitrogen atom, organization in molecular networks and were matched with the IQAMDB database.[20] The annotated alkaloids stepholidine (**3**), reticuline (**5**), coclaurine (**1**) and *N*-methylcoclaurine (**2**) were directly indicated by the IQAMDB database, while the others were confirmed through manual interpretation of MS/MS spectra with comparison with previously published data.[18] The MS/MS data, when visualized using molecular networks, allowed the observation of the grouping of the nodes according to the types of isoquinoline skeletons found in the samples. The molecular network generated (Figure 2) presented a majority cluster with seven nodes, and three smaller clusters, but with little or no significance in the chemical composition of interest of the sample.

Initially, our analysis approach was validated using the stepholidine alkaloid at *m/z* 328.1533 $[M + H]^+$ (**3**, $C_{19}H_{21}NO_4$, –4.57 ppm), which was used as a seed in this study. This pattern (**3**), previously isolated by our research group (Figures S3-S11, Supplementary Information (SI) section), was chosen as the seed due to its prior identification in the same species.[7] Directly connected to this node, *m/z* 342.1707 $[M + H]^+$ was annotated as the isocorypalmine alkaloid (**13**, $C_{20}H_{23}NO_4$, 0.58 ppm) (Figure S21, SI section), since they both showed a very similar fragmentation profile.[37] Another group of alkaloids was identified in the same main cluster, which were of the benzylisoquinoline type (Figure 2) and annotated as petaline (*m/z* 328.1925 $[M + H]^+$, **14**, $C_{20}H_{26}NO_3^+$, 3.96 ppm) (Figure S14),[38] reticuline (*m/z* 330.1705 $[M + H]^+$, **5**, $C_{19}H_{23}NO_4$, 0.00 ppm)

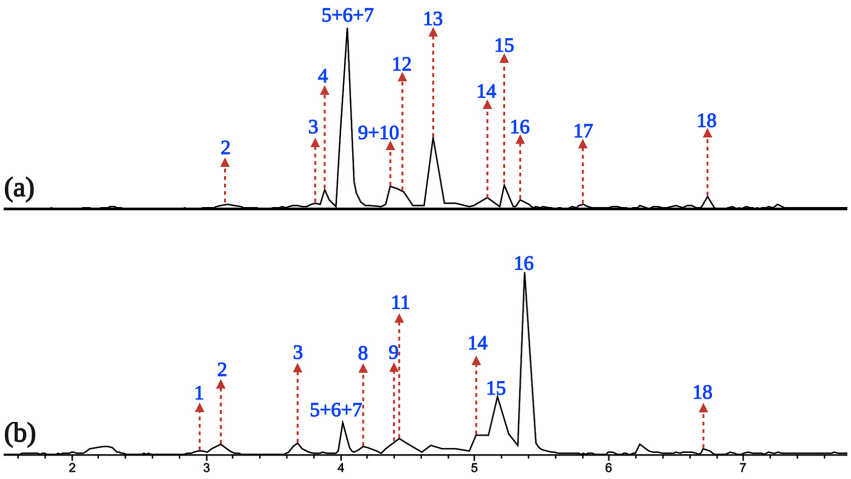

Figure 1. Total ion chromatogram of *F. longifolia* from the alkaloid fraction of leaves (a) and branches (b).

(Figure S13), reticuline *N*-oxide (*m/z* 346.1658 $[M + H]^+$, **4**, $C_{19}H_{23}NO_5$, 1.55 ppm) (Figure S12, SI section),[1] *N*-methylcoclaurine (*m/z* 300.1611 $[M + H]^+$, **2**, $C_{18}H_{21}NO_3$, 3.99 ppm) (Figure S2),[1] oblongine (*m/z* 314.1780 $[M + H]^+$, **6**, $C_{19}H_{24}NO_3^+$, 7.63 ppm) (Figure S14, SI section),[39] armepavine (*m/z* 314.1763 $[M + H]^+$, **7**, $C_{19}H_{24}NO_3$, 2.22 ppm) (Figure S15, SI section)[40] and coclaurine (*m/z* 286.1457 $[M + H]^+$, **1**, $C_{17}H_{19}NO_3$, 4.89 ppm) (Figure S1, SI section).[41] The fragmentation spectra of compounds **1**, **2**, **4**-**7**, **13** and **14** showed very similar fragmentation profiles, in which the dominant losses were the exit of the southern portion of the molecule (ring C, substituted benzyl) and the northern portion (ring A, substituted isoquinoline nucleus), in which the substitution patterns of aromatic rings A and C were deduced by the masses of the fragments, as well as by characteristic losses previously described (Table 1).[18]

Surprisingly, peak **8** eluted in 4.3 min (Figure 1), presented *m/z* 462.2146 $[M + H]^+$, which was located in the molecular network near the nodes of benzylisoquinoline alkaloids, especially near compound **1** (Figure 2). The molecular formula $C_{24}H_{31}NO_8$ (4.11 ppm) was deduced from the exact mass, and this information in conjunction with the initial neutral loss of 162 Da (– hexoside, *m/z* 462 → *m/z* 300) indicated the presence of a glycosylated isoquinoline alkaloid. Then, characteristic cleavage of the amino portion was observed at 17 Da ($–NH_3$) (*m/z* 300 → *m/z* 283) with subsequent losses of 32 Da ($–CH_2OH$) (*m/z* 283 → *m/z* 251) and 28 Da (–CO) (*m/z* 251 → *m/z* 223), which is characteristic of adjacent hydroxyl and methoxyl groups on ring A. Furthermore, compound **8** produced diagnostic fragment ions typical of the benzylisoquinoline skeleton at *m/z* 178 and 121, indicating vicinal methoxyl and hydroxyl groups in ring A and methoxyl group in ring C.[42] Glycosylated alkaloids are rarely found in natural sources; however, isoquinoline alkaloids with glucose portions in their structure have been reported in the literature.[43]

Among the substances annotated above, only stepholidine (**3**) is described in *F. longifolia*.[7] Thus, the other substances identified by LC-MS/MS and via molecular networking are reported for the first time for the target species of this study and for the genus *Fusaea*. In addition, some of these alkaloids already have proven pharmacological properties. The alkaloid stepholidine (**3**), which was isolated from *Annona cherimola*, is a promising neuroprotector,[44] and isocorypalmine (**13**) has an insecticidal effect.[45] The compounds reticuline (**5**) and

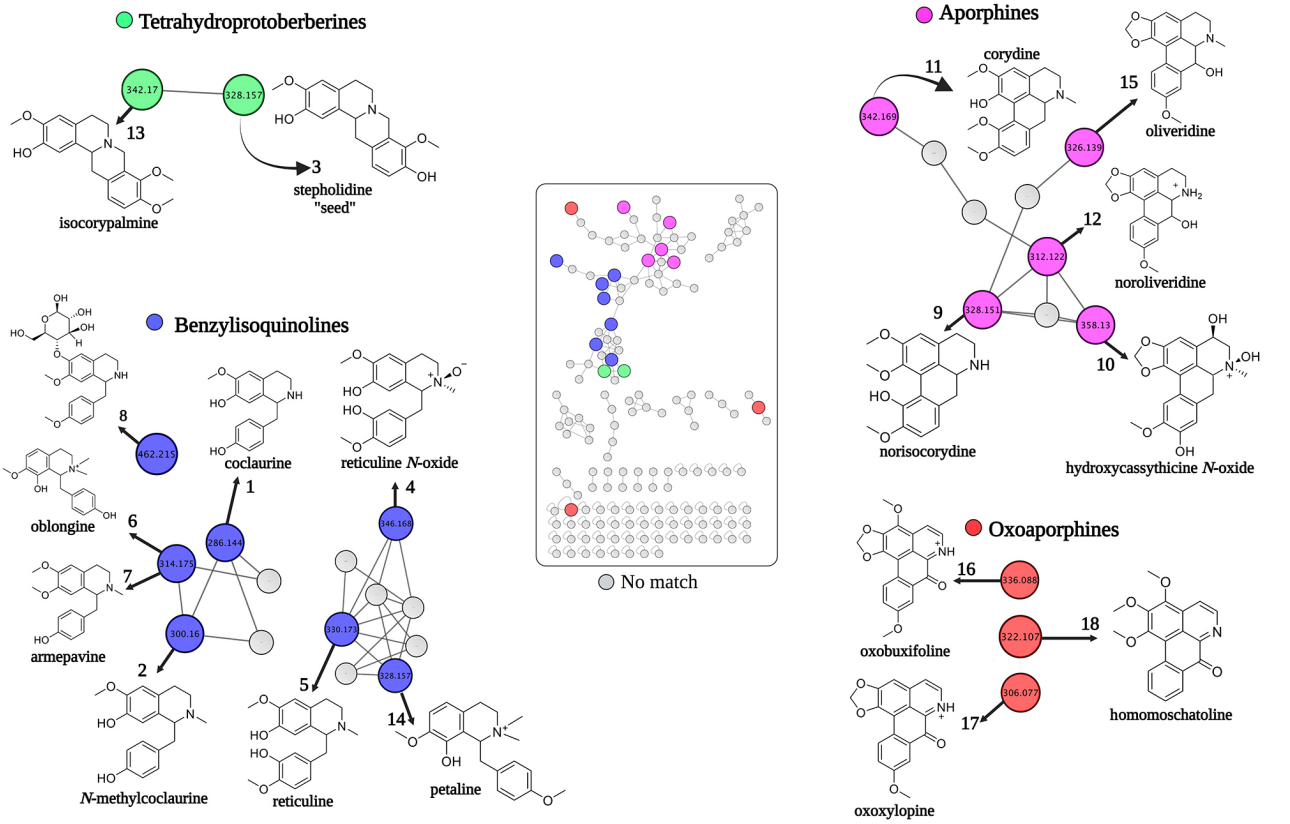

Figure 2. Annotation of the molecular network of the alkaloid-rich fraction derived from the methanolic extract of branches and leaves of *F. longifolia*, which shows benzylisoquinolines (blue knots), tetrahydroisoquinolines (green knots), aporphines (pink knots) and oxoaporphines (red knots). Nodes in gray, without *m/z* description, could not be annotated.

N-methylcoclaurine (**2**), isolated from *Peumus boldus* (Monimiaceae), show promising activities of inhibition of butyrylcholinesterase, an enzyme of the cholinesterase group.[46] The natural product, coclaurine (**1**), isolated from *Annona squamosa*, has cytotoxicity against colon cancer cells (HCT116), human breast cancer cells (MCF-7) and human liver cancer cells (HepG2).[2]

From the analysis of the molecular network, other nodes of the main cluster that correspond to aporphine alkaloids were annotated, which were corroborated based on fragmentation patterns.[47] When analyzing the MS/MS spectra, neutral losses of 17 Da ($-NH_3$) (**9** and **12**) and 31 Da ($-NH_2CH_3$) (**11** and **15**) were observed. In addition, the fragmentation pathways for compounds **12**, **15** and **10** indicated neutral losses of methanal ($-CH_2O$, 30 Da) with concomitant elimination of carbon monoxide (–CO, 28 Da), which is characteristic of the presence of a methylene dioxide group in the rings A or D.[47] Compounds **9** and **11**, presented pathways consistent with aporphines with adjacent methoxyl groups in the ring A, which establish competitive radical losses of 31 Da ($\bullet OCH_3$) and 15 Da ($\bullet CH_3$). Therefore, these ions may correspond to the alkaloids norisocorydine **9** (*m/z* 328.1568 $[M + H]^+$, $C_{19}H_{21}NO_4$, 5.78 ppm) (Figure S17, SI section),[48] corydine **11** (*m/z* 342.1719 $[M + H]^+$, $C_{20}H_{23}NO_4$, 4.09 ppm) (Figure S19, SI section),[49] noroliveridine **12** (*m/z* 312.1237 $[M + H]^+$, $C_{18}H_{17}NO_4$, 0.64 ppm) (Figure S20, SI section),[50] oliveridine **15** (*m/z* 326.1405 $[M + H]^+$, $C_{19}H_{19}NO_4$, 4.3 ppm) (Figure S23, SI section)[51] and hydroxycassythicine *N*-oxide **10** (*m/z* 358.1301 $[M + H]^+$, $C_{19}H_{20}NO_6$, 3.07 ppm) (Figure S18, SI section).[52]

Other alkaloids were also dereplicated, whereby the MS/MS spectra of protonated molecules at *m/z* 306.0770 $[M + H]^+$ (**17**, $C_{18}H_{11}NO_4$, 1.30 ppm) (Figure S25, SI section), 322.1082 $[M + H]^+$ (**18**, $C_{19}H_{15}NO_4$, 0.93 ppm) (Figure S26, SI section) and 336.0876 $[M + H]^+$ (**16**, $C_{19}H_{13}NO_5$, 1.19 ppm) (Figure S24, SI section) were attributed to fragment pathways for oxoaporphine alkaloids, as described above[53] for oxoxylopine alkaloids,[54] homomoschatoline[55] and oxobuxifoline,[56] respectively. The aporphine and oxoaporphine alkaloids mentioned above are reported for the first time in the species discussed in this study and in the genus *Fusaea*.

Aporphine **12** (noroliveridine) is found in *Duguetia spixiana*,[50] and oliveridine (**15**) has been isolated from *Duguetia vallicola* and *Garcinia parvifolia* (Clusiaceae); both studies showing anti-*P. falciparum* activity.[51,57] Studies with the alkaloid oxoxylopine (**17**), an oxoaporphine, exhibited cytotoxicity against U251 (brain tumor cell line) and HEOG2 (hepatocellular carcinoma cell line) with an IC_{50} of 4 and 2.5 μg mL^{-1}, respectively.[58] The oxoaporphine homomoschatoline (**18**) showed significant *in vitro* lethality against *Artemia franciscana* larvae.[59] These literature reports reinforce that the isoquinoline

Table 1. Isoquinoline alkaloids in *F. longifolia* annotated using LC-MS/MS

Compound	Class	t_R / min	Plant organ	$[M + H]^+$	Chemical formula	Error / ppm
Coclaurine (**1**)	B	2.9	l	286.1457	$C_{17}H_{19}NO_3$	+4.89
N-Methylcoclaurine (**2**)	B	3.1	b/l	300.1611	$C_{18}H_{21}NO_3$	+3.99
Stepholidine (**3**)	T	3.8	b/l	328.1533	$C_{19}H_{21}NO_4$	-4,57
Reticuline *N*-oxide (**4**)	B	3.9	b	346.1658	$C_{19}H_{23}NO_5$	+1.55
Reticuline (**5**)	B	4.1	b/l	330.1705	$C_{19}H_{23}NO_4$	0.00
Oblongine (**6**)	B	4.1	b/l	314.1780	$C_{19}H_{24}NO_3$	+7.63
Armepavine (**7**)	B	4.2	b/l	314.1763	$C_{19}H_{24}NO_3$	+2.22
(**8**)	–	4.3	l	462.2146	$C_{24}H_{31}NO_8$	–
Norisocorydine (**9**)	A	4.4	b/l	328.1568	$C_{19}H_{21}NO_4$	+5.78
Hydroxycassythicine *N*-oxide (**10**)	A	4.4	b	358.1301	$C_{19}H_{20}NO_6$	+3.07
Corydine (**11**)	A	4.4	b/l	342.1719	$C_{20}H_{23}NO_4$	+4.09
Noroliveridine (**12**)	A	4.5	b	312.1237	$C_{18}H_{17}NO_4$	+0.64
Isocorypalmine (**13**)	T	4.7	b/l	342.1707	$C_{20}H_{23}NO_4$	+0.58
Petaline (**14**)	B	5.0	b/l	328.1925	$C_{20}H_{26}NO_3$	+3.96
Oliveridine (**15**)	A	5.3	b/l	326.1405	$C_{19}H_{19}NO_4$	+4.3
Oxobuxifoline (**16**)	O	5.7	b/l	336.0876	$C_{19}H_{13}NO_5$	+1.19
Oxoxylopine (**17**)	O	5.8	b	306.0770	$C_{18}H_{11}NO_4$	+1.30
Homomoschatoline (**18**)	O	6.1	b/l	322.1082	$C_{19}H_{15}NO_4$	+0.93

Class: aporphine (A), benzylisoquinoline (B), tetrahydroprotoberberine (T) oxoaporphine (O); t_R: retention time; plant organ: b (branches), l (leaves).

alkaloids annotated in *F. longifolia* are promising for anti-*Plasmodium* tests.

Anti-*Plasmodium* activity

The alkaloid fractions of *F. longifolia* were sent for *in vitro* assays against strains of *P. falciparum* (FRC3), with quinine as the control. The alkaloid fractions showed high anti-*Plasmodium* activity and exhibited an IC_{50} of 2.42 μg mL^{-1} (branches) and 1.60 μg mL^{-1} (leaves), which was similar to the action of quinine, which has an IC_{50} of 1.24 μg mL^{-1}.

Several reports in the literature contribute and strengthen these results, such as the studies by Boyom *et al*.[60] in which the *in vitro* antimalarial activity of extracts (MeOH) from the stem bark of *Xylopia africana* (Annonaceae) against W2 resistant strains of *P. falciparum* showed significant results with an IC_{50} of 1.07 μg mL^{-1}. Extracts (MeOH) from leaves of *Guatteria amplifolia* (Annonaceae) have been proven to be quite active against D2 strains of *P. falciparum*, with an IC_{50} of 1.5 μg mL^{-1}.[61] On the other hand, the alkaloid fractions of seeds of *Anonidium mannii* (Annonaceae) were active with an IC_{50} value of 2.4 μg mL^{-1} against strain W2 of *P. falciparum.*[60]

The significant activity shown in these results can be attributed to the alkaloids that are present in the fractions, and antiplasmodic activity has already been reported in the literature. In the search for new antimalarial agents, Levrier *et al*.[62] isolated anonaine, an alkaloid benzylisoquinoline already reported in *F. longifolia*,[6] from the leaves of *Goniothalamus australis* (Annonaceae) and revealed a significant anti-*Plasmodium* effect against strain 3D7 of *P. falciparum*, with an IC_{50} of 2.7 μg mL^{-1}.

In this sense, the results lead to believe that the promising anti-*Plasmodium* activity reported in the present study is attributed to the isoquinoline alkaloid constituents present in extracts of species of the Annonaceae family. This highlights the importance of prospecting and conducting anti-*Plasmodium* tests with this class of substances.

Cytotoxic activity

The alkaloid fractions of the leaves and branches of *F. longifolia* were submitted to the cell viability test using the healthy human lung fibroblast lineage (MRC-5). The samples were considered active when they presented an IC_{50} higher than 50 μg mL^{-1}; therefore, the higher the IC_{50} value, the better the result in this situation, with samples being less toxic to healthy cells. As such, the alkaloid fractions showed satisfactory results, with moderate cytotoxic activity,[63] and mean IC_{50} values of > 50 μg mL^{-1}. Doxorubicin was used as the positive control and exhibited an IC_{50} of 3.18 μg mL^{-1}.

Previous studies[64] show that extracts and alkaloids of species of the Annonaceae family demonstrate low cytotoxicity against the non-cancerous cell line MRC-5. One example of this is the recent study by Costa *et al*.,[1] who isolated nine alkaloids (isoquinoline derivatives) from *Diclinanona calycina* (Annonaceae) bark and these were evaluated against non-cancerous cell lines. In the promising results, five alkaloids (thalifoline, reticuline, reticuline N_{β}-oxide, reticuline N_{α}-oxide and bisnorargemonine) showed low cytotoxicity against the MRC-5 cell line (> 25 μg mL^{-1}). Another example is the alkaloid vincosamide, isolated from *Psychotria leiocarpa* (Rubiaceae), which showed moderate cytotoxicity with an IC_{50} of 50 μg mL^{-1}, but with a high reduction in infectious diseases such as dengue. Despite vincosamide's moderate cytotoxicity, these results indicate this compound as a potential anti-dengue agent.[63] Thus, the importance of finding efficient results with alkaloid fractions that are not toxic to healthy cells becomes a relevant factor for the continuity of studies with the species *F. longifolia*.

Molecular docking

The dereplicated compounds were subjected to molecular docking assays against four different proteins from *P. falciparum*. The first step of the molecular docking process was redocking, a procedure that involves the removal and repositioning of the ligand at the protein binding site, which allows the evaluation of the reproduction capacity and validity of the results obtained. The redocking results of dm-P*f*DHFR (PDB ID: 1J3J), P*f*DHODH (PDB ID: 1TV5), qm-P*f*DHFR (PDB ID: 1J3K) and P*f*PNP (PDB ID: 5ZNC), were –0.4912, –0.5461, –0.8038 and –0.9917 Å, respectively (Figure 3). These values are considered acceptable for the redocking procedure (RMSD < 2), and suggest that the model is able to reproduce the correct conformation of the ligand. These results are in agreement with virtual screening approaches of *P. falciparum* developed with these models.[17]

Next, the annotated alkaloids were similarly tested at the binding site of the target enzymes. The binding energy of the alkaloids (**1**-**18**) in relation to P*f*PNP (PDB ID: 5ZNC) ranged from –6.8 to –10.1 kcal mol^{-1}, and alkaloids **5**, **1**, **17** and **16** represented higher affinities when compared to quinine. Notably, the oxoaporphine alkaloid oxoxylopine **17** (–10.1 kcal mol^{-1}) had a higher score than the reference drug (quinine, –8.5 kcal mol^{-1}, RMSD < 2). The interactions observed for alkaloid **17** showed that the oxygen of the *O*-methyl group established a hydrogen interaction with

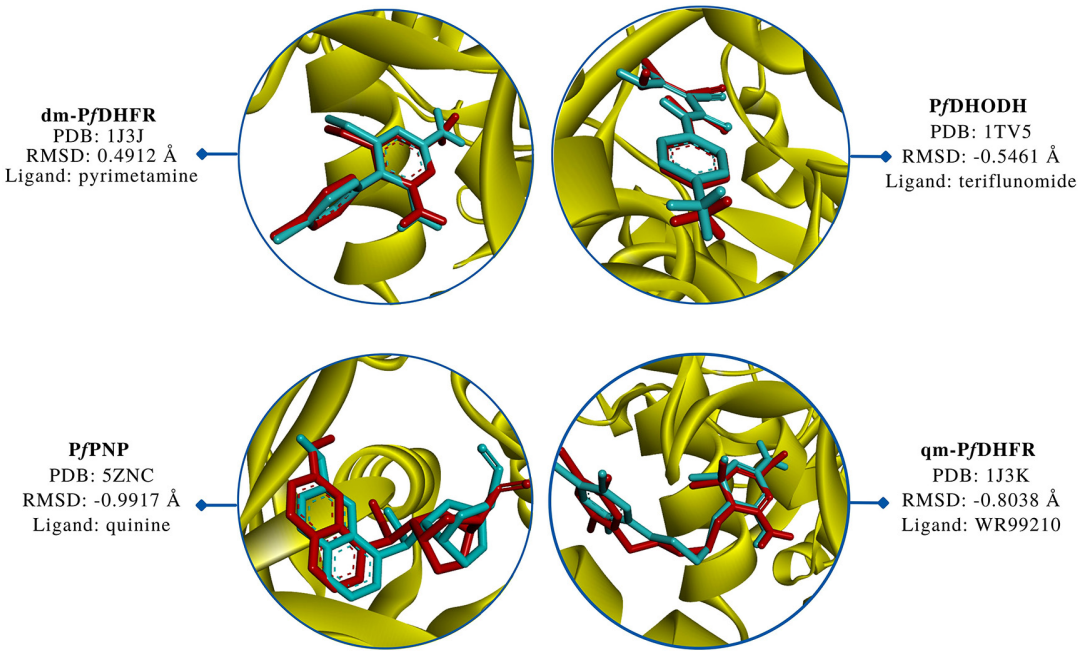

Figure 3. Overlapping redocked ligands (in red) with the ligand-bound conformations of the X-ray crystal structures (blue).

Ser91, while the nitrogen of the *N*-methyl group formed a hydrogen interaction with the residue Asp206, these being residues of the catalytic site.[65] In addition, π-alkyl interactions were observed in the rings A and C with Met183, rings B and E with Val181 and rings D and E with Pro209. Whereas, π-π stacking interaction in ring D with Tyr160 and Trp212 was also shown (Figure 4 and Table S1, SI section).

The binding affinity of teriflunomide for P*f*DHODH (PDB ID: 1TV5) was –9.6 kcal mol^{-1}, while alkaloids were bound to the same protein with binding energies ranging from –5.6 to –9.8 kcal mol^{-1}. Alkaloids **12**, **10** and **18** had higher protein binding affinities, and the aporphine alkaloid hydroxycassythicine *N*-oxide **10** (–9.8 kcal mol^{-1}, RMSD < 2) was more effective in binding-protein affinity when compared to teriflunomide (Table S2, SI section). The interactions for alkaloid **10** showed that the oxygen atom of the methylenedioxy group participated in hydrogen interaction with Cys276, and hydrogen interaction between the hydroxyl oxygen of the aromatic ring C with Ser505, and a π-alkyl interaction was observed for the methylenedioxy group with Ile263, which are considered key residues of the active site.[66]

The co-crystallized ligand WR99210 for the quadruple mutant qm-P*f*DHFR (PDB ID: 1J3K) has a binding affinity of –8.3 kcal mol^{-1}, and the binding energy of alkaloids in relation to the protein are between –7.1 and –9.6 kcal mol^{-1}. Among these, alkaloids **14**, **4**-**6**, **9**-**15**, **17**-**16** have higher affinities compared to WR99210, and alkaloid **17** was superior with –9.6 kcal mol^{-1}, RMSD < 2 (Table S3, SI section). The interactions observed for the alkaloid oxoxylopine **17** showed that the oxygen atom of the methylenedioxy group participated in hydrogen interaction with Tyr170, while the nitrogen of the *N*-methyl group of ring E and the carbonyl of ring B formed hydrogen interactions with the residue Ser111, as well as the oxygen of the *O*-methyl group of the ring A, which participated in hydrogen interaction with Ser167. Alkaloid **17** also showed π-π stacking interaction in rings D and A with the catalytic residue Phe58 and Leu40.[15]

Regarding dm-P*f*DHFR (PDB ID: 1J3J), alkaloids **4**, **12**, **15**, **17** and **16** presented better values, between –8.0 to –9.4 kcal mol^{-1}. Overall, once more the alkaloid oxoxylopine **17** stood out by showing binding energy of –9.4 kcal mol^{-1}, RMSD < 2, while pyrimetamine was bound to the protein with binding energy of –7.9 kcal mol^{-1} (Table S4, SI section). The interactions observed for alkaloid **17** showed that the carbonyl of ring B formed a hydrogen interaction with Ser111, and the nitrogen of the *N*-methyl group of ring E formed a hydrogen interaction with the residue of the active site Asn108.[66] In addition, π-alkyl interactions were observed in ring B with Leu46, and in rings C and D with Ala16.

When the docking analyses are considered, the π-π interactions can be seen dominant for the oxoaporphine alkaloid oxoxylopine **17**, which favors the activity in the enzymes P*f*PNP, qm-P*f*DHFR and dh-P*f*DHFR. The binding site of ligands to the enzymes is strongly hydrophobic and coated by aromatic residues, thus suggesting an increase in potency via the accommodation of compounds with interactions favored by π-π stacking at

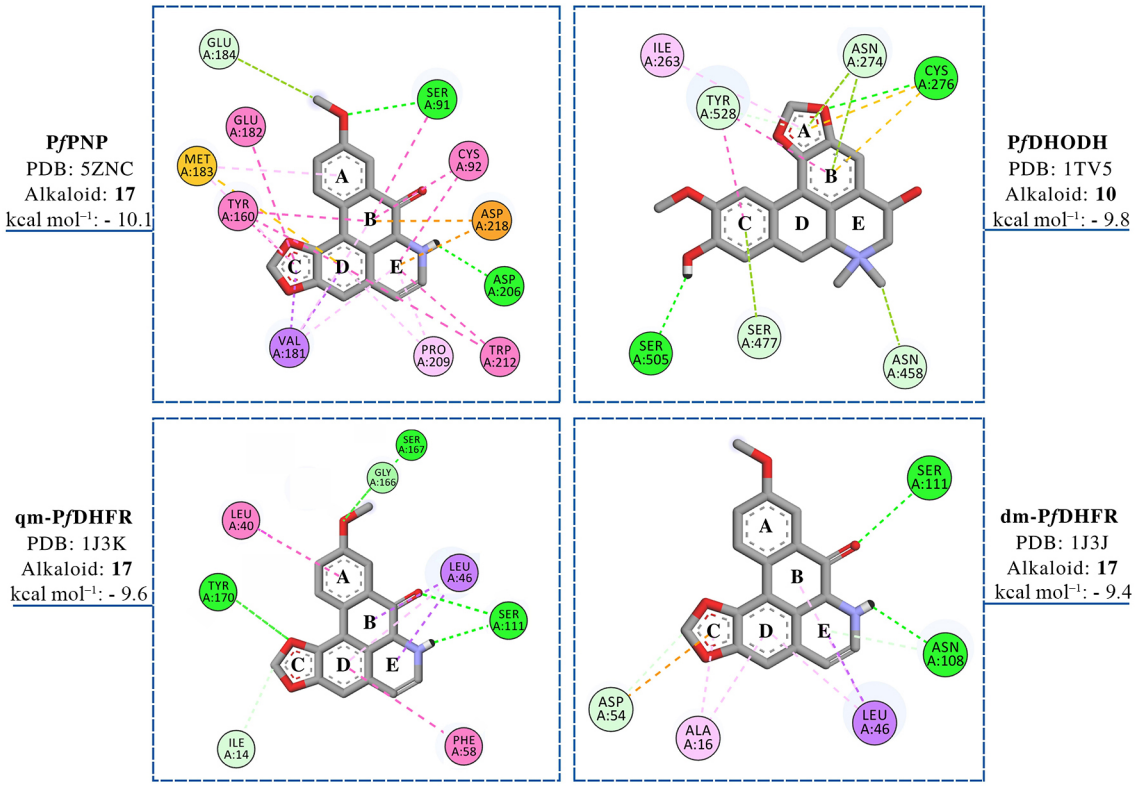

Figure 4. General interactions of lower energy alkaloids with amino acid residues in the binding site region of the *P. falciparum* protein complex.

the binding site. Oxoaporphines are aromatic and planar structures, which makes their structure more rigid and with limitations of degrees of freedom. These characteristics guarantee a more effective coupling; therefore, docking is favored. The literature shows that oxoaporphine alkaloids, such as liriodenine and lysicamine have potential *in vitro* against *P. falciparum*.[67] Alkaloid oxoxylopine (**17**) showed cytotoxicity against U251e and HEPG2, with IC_{50} values of 4 and 2.5 μg mL^{-1}, respectively.[59] Oxoxylopine also has antimicrobial activity[68] and antiplatelet activity.[69]

The docking study provided a very useful tool for interpreting the results of the *in vitro* inhibitory activity of the alkaloid fractions against *P. falciparum*, and indicated that the dereplicated alkaloids under investigation may be able to bind effectively to the active site of target proteins that are vital to the parasite. These observations, along with key interactions in the docking analyses, may be useful when planning new antimalarials. Overall, the proposed approach suggests the usefulness of alkaloids **10** and **17** as a suitable model that can be used as a prototype for the design of new therapeutic agents that are capable of disrupting the crucial functions of *P. falciparum* enzymes. Given this scenario, the search for new antimalarial drugs is essential and natural products have played an important role in the discovery of molecules with chemotherapeutic activity for the treatment of human diseases.

Conclusions

The combination of manual interpretation of LC-MS/MS spectra with the analysis of molecular network data allowed the dereplication of 18 isoquinoline alkaloids in *F. longifolia* species and, among these, an unknown glycosylated alkaloid was annotated. Among the known alkaloids, 17 are described for the first time in *F. longifolia*; nevertheless, NMR analysis is needed to identify the new molecules. In addition, these findings help in the tedious isolation of constituents, thus minimizing costs and optimizing the time spent in this process, and is a useful strategy to avoid the reisolation of compounds already described in the literature.

The biological tests confirmed moderate cytotoxic activity in healthy MRC-5 cells and the anti-*Plasmodium* potential of the species *F. longifolia* was observed for the branches (IC_{50} of 2.42 μg mL^{-1}) and the leaves (1.60 μg mL^{-1}), which can be associated with synergism of bio-isoquinolinic alkaloids present in the fractions. Together with this, this study showed a good correlation of the experimental values of IC_{50} with the *in silico* activity of molecular docking, thus providing a better understanding of the inhibitory potential of dereplicated alkaloids, especially oxoaporphine (**17**) and aporphine (**10**) alkaloids, as new sources of protein inhibitors vital to the

parasite *P. falciparum*. These findings deserve attention and stimulate the intensification of investigations of the full pharmacological potential of these compounds. The results obtained contribute to the knowledge of natural products of the Annonaceae family, as well as highlight *F. longifolia* as a source of bioactive substances.

Supplementary Information

Supplementary information (NMR spectra, high resolution mass spectra and ligand interactions table) is available free of charge at https://jbcs.sbq.org.br as a PDF file.

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Author Contributions

AAXA was responsible for investigation, data curation and writing the original draft; CVAS, MSA and WHPP for data curation; LSM and FMAS for data curation and validation; WMM, DPB and GCM for formal analysis and validation; ZMS, EVC and JFT for formal analysis, validation, writing-review and editing; HHFK for conceptualization, data curation, formal analysis, funding acquisition, investigation, project administration, resources, validation, writing the original draft, review and editing.

References

1. Costa, E. V.; Soares, L. D. N.; Chaar, J. D. S.; Silva, V. R.; Santos, L. D. S.; Koolen, H. H. F.; Bezerra, D. P.; *Molecules* **2021**, *26*, 3714. [Crossref]
2. Al-Ghazzawi, A. M.; *BMC Chem*. **2019**, *13*, 13. [Crossref]
3. Abdul, W. S. M.; Jantan, I.; Haque, M. A.; Arshad, L.; *Front. Pharmacol.* **2018**, *9*, 661. [Crossref]
4. Sharma, G.; Rana, D.; Sundriyal, S.; Sharma, A.; Panwar, P.; Mahindroo, N.; *S. Afr. J. Bot.* **2023**, *155*, 154. [Crossref]
5. Chatrou, L. W.; He, P.; *Brittonia* **1999**, *51*, 18. [Crossref]
6. Braz Fo, R.; Gabriel, S. J.; Gomes, C. M. R.; Gottlieb, O. R.; Bichara, M. D. G. A.; Maia, J. G. S.; *Phytochem*. **1976**, *15*, 1187. [Crossref]
7. Tavares, J. F.; Barbosa-Filho, J. M.; da Silva, M. S.; Maia, J. G. S.; da-Cunha, E. V. L.; *Rev. Bras. Farmacogn*. **2005**, *15*, 115. [Crossref]
8. Bay, M.; de Oliveira, J. V. S.; Sales Jr., P. A.; Murta, S. M. F.; dos Santos, A. R.; Bastos, I. S.; Orlandi, P. P.; de Sousa Jr., P. T.; *Chem. Biodiversity* **2019**, *16*, e1900359. [Crossref]
9. Ferreira-Silva, M. M.; Carlos, A. M.; Resende, G. A. D.; *Medicine* **2021**, *2*, 7. [Crossref]
10. World Health Organization (WHO); *World Malaria Report 2022*, https://www.who.int/publications/i/item/9789240064898, accessed on 15 December, 2023.
11. Venkatesan, P.; *Lancet Microbe* **2022**, *3*, 251. [Crossref]
12. Reed, M. B.; Saliba, K. J.; Caruana, S. R.; Kirk, K.; Cowman, A. F.; *Nature* **2000**, *403*, 906. [Crossref]
13. Chokchaisiri, R.; Chaichompoo, W.; Chalermglin, R.; Suksamrarn, A.; *Rec. Nat. Prod.* **2015**, *9*, 243. [Link] accessed in December 2023
14. Phillips, M. A.; Rathod, P. K.; *Infect. Disord.: Drug Targets* **2010**, *10*, 226. [Crossref]
15. Yuthavong, Y.; Yuvaniyama, J.; Chitnumsub, P.; Vanichtanankul, J.; Chusacultanachai, S.; Tarnchompoo, B.; Vilaivan, T.; Kamchonwongpaisan, S.; *Parasitology* **2005**, *130*, 249. [Crossref]
16. Cui, H.; Ruda, G. F.; Carrero-Lérida, J.; Ruiz-Pérez, L. M.; Gilbert, I. H.; González-Pacanowska, D.; *Eur. J. Med. Chem.* **2010**, *45*, 5140. [Crossref]
17. Kyei, L. K.; Gasu, E. N.; Ampomah, G. B.; Mensah, J. O.; Borquaye, L. S.; *J. Chem*. **2022**, *2022*, ID 5314179. [Crossref]
18. de Lima, B. R.; da Silva, F. M. A.; Soares, E. R.; de Almeida, R. A.; da Silva-Filho, F. A.; Barison, A.; Costa, E. V.; Kollen, H. H. F.; de Souza, A. D. L.; Pinheiro, M. L. B.; *J. Braz. Chem. Soc*. **2020**, *31*, 79. [Crossref]
19. Agnès, S. A.; Okpekon, T.; Kouadio, Y. A.; Jagora, A.; Bréard, D.; Costa, E. V.; da Silva, F. M. A.; Koolen H. H. F.; Le Ray-Richomme, A. M.; Richomme, P.; Champy, P.; Beniddir, M. A.; Le Pogam, P.; *Sci. Data* **2022**, *9*, 270. [Crossref]
20. Costa, E. V.; Pinheiro, M. L. B.; Xavier, C. M.; Silva, J. R. A.; Amaral, A. C. F.; Souza, A. D. L.; Barison, A.; Campos, F. R.; Ferreira, A. G.; Machado, G. M. C.; Leon, L. L. P.; *J. Nat. Prod.* **2006**, *69*, 292. [Crossref]
21. *Data Analysis*, version 4.2; Bruker Daltonik GmbH, Bremen, Germany, 2013.

22. Wang, M.; Carver, J. J.; Phelan, V. V.; Sanchez, L. M.; Garg, N.; Peng, Y.; Nguyen, D. D.; Watrous, J.; Kapono, C. A.; Tal Luzzatto-Knaan, T.; Carla Porto, C.; Amina Bouslimani, A.; Melnik, A. V.; Meehan, M. J.; Liu, W.-T.; Crüsemann, M.; Boudreau, P. D.; Esquenazi, E.; Sandoval-Calderón, M.; Kersten, R. D.; Pace, L. A.; Quinn, R. A.; Duncan, K. R.; Hsu, C.-C.; Floros, D. J.; Gavilan, R. G.; Kleigrewe, K.; Northen, T.; Dutton, R. J.; Parrot, D.; Carlson, E. E.; Aigle, B.; Michelsen, C. F.; Jelsbak, L.; Sohlenkamp, C.; Pevzner, P.; Edlund, A.; McLean, J.; Piel, J.; Murphy, B. T.; Gerwick, L.; Liaw, C.-C.; Yang, Y.-L.; Humpf, H.-U.; Maansson, M.; Keyzers, R. A.; Sims, A. C.; Johnson, A. R.; Sidebottom, A. M.; Sedio, B. E.; Klitgaard, A.; Larson, C. B.; Boya, P. A. A.; Torres-Mendoza, D.; Gonzalez, D. J.; Silva, D. B.; Marques, L. M.; Demarque, D. P.; Pociute, E.; O'Neill, E. C.; Briand, E.; Helfrich, E. J. N.; Granatosky, E. A.; Evgenia Glukhov, E.; Florian Ryffel, F.; Houson, H.; Mohimani, H.; Kharbush, J. J.; Zeng, Y.; Vorholt, J. A.; Kurita, K. L.; Charusanti, P.; McPhail, K. L.; Nielsen, K. F.; Vuong, L.; Elfeki, M.; Traxler, M. F.; Engene, N.; Koyama, N.; Vining, O. B.; Baric, R.; Silva, R. R.; Mascuch, S. J.; Tomasi, S.; Jenkins, S.; Macherla, V.; Hoffman, T.; Agarwal, V.; Williams, P. G.; Dai, J.; Neupane, R.; Gurr, J.; Rodríguez, A. M. C.; Lamsa, A.; Zhang, C.; Dorrestein, K.; Duggan, B. M.; Almaliti, J.; Allard, P.-M.; Phapale, P.; Nothias, L.-F.; Alexandrov, T.; Litaudon, M.; Wolfender, J.-L.; Kyle, J. E.; Metz, T. O.; Peryea, T.; Nguyen, D.-T.; VanLeer, D.; Shinn, P.; Jadhav, A.; Müller, R.; Waters, K. M.; Wenyuan Shi, W.; Liu, X.; Zhang, L.; Knight, R.; Jensen, P. R.; Palsson, B. Ø.; Pogliano, K.; Linington, R. G.; Gutiérrez, M.; Lopes, N. P.; Gerwick, W. H.; Moore, B. S.; Dorrestein, P. C.; Bandeira, N.; *Nat. Biotechnol*. **2016**, *34*, 828. [Crossref]
23. Chambers, M. C.; Maclean, B.; Burke, R.; Amodei, D.; Ruderman, D. L.; Neumann, S.; Gatto, L.; Fischer, B.; Pratt, B.; Egertson, J.; Hoff, K.; Kessner, D.; Tasman, N.; Shulman, N.; Frewen, B.; Baker, T. A.; Brusniak, M.-Y.; Paulse, C.; Creasy, D.; Flashner, L.; Kani, K.; Moulding, C.; Seymour, S. L.; Nuwaysir, L. M.; Lefebvre, B.; Kuhlmann, F.; Roark, J.; Rainer, P.; Detlev, S.; Hemenway, T.; Huhmer, A.; Langridge, J.; Connolly, B.; Chadick, T.; Holly, K.; Eckels, J.; Deutsch, E. W.; Moritz, R. L.; Katz, J. E.; Agus, D. B.; MacCoss, M.; Tabb, D. L.; Parag Mallick, P.; *Nat. Biotechnol*. **2012**, *30*, 918. [Crossref]
24. Shannon, P.; Markiel, A.; Ozier, O.; Baliga, N. S.; Wang, J. T.; Ramage, D.; Ideker, T.; *Genome Res*. **2003**, *13*, 2498. [Crossref]
25. Global Natural Product Social Molecular Networking (GNPS), https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=84e8faf2a737496db10094eb0bf4b133, accessed in December 2023.
26. Ljungström, I.; Moll, K.; Perlmann, H.; Scherf, A.; Wahlgren, M.; *Methods in Malaria Research;* MR4/ATCC: Manassas, VA, USA, 2008. [Crossref]
27. Lopes, S. C. P.; Blanco, Y. C.; Justo, G. Z.; Nogueira, P. A.; Rodrigues, F. L.; Goelnitz, U.; Costa, F. T. M.; *Antimicrob. Agents Chem*. **2009**, *53*, 2149. [Crossref]
28. *GraphPad Prism*, 8.2; GraphPad Software, USA, 2019.
29. Ahmed, S. A.; Gogal Jr., R. M.; Walsh, J. E.; *J. Immunol. Methods* **1994**, *170*, 211. [Crossref]
30. Santos, L. S.; Silva, V. R.; Menezes, L. R. A.; Soares, M. B. P.; Costa, E. V.; Bezerra, D. P.; *Oxid. Med. Cell. Longevity* **2017**, *2017*, ID 7126872. [Crossref]
31. Santos, J. O.; Pereira, G. R.; Brandão, G. C.; Borgati, T. F.; Arantes, L. M.; Paula, R. C. D.; Oliveira, A. B. D.; *J. Braz. Chem. Soc*. **2016**, *27*, 551. [Crossref]
32. *MarvinSketch*, 17.1.2.0; ChemAxon, Budapest, 2017. [Link] accessed in December 2023
33. *MOPAC2016*; Stewart Computational Chemistry, Colorado Springs, CO, USA, 2016. [Link] accessed in December 2023
34. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; *J. Comput. Chem*. **2009**, *30*, 2785. [Crossref]
35. Trott, O.; Olson, A. J.; *J. Comput. Chem*. **2009**, *31*, 455. [Crossref]
36. *Discovery Studio Visualizer*, v 16.1.0; AccelrysInc, San Diego, CA, USA, 2016. [Link] accessed in December 2023
37. Nardelli, V. B.; de Souza, C. A. S.; Chaar, J. S.; Koolen, H. H. F.; da Silva, F. M. A.; Costa, E. V.; *Biochem. Syst. Ecol*. **2021**, *94*, 104206. [Crossref]
38. Tacić, D.; Wannigama, G. P.; Cassels, B. K.; Cavé, A.; *J. Nat. Prod*. **1987**, *5*, 518. [Crossref]
39. Guo, K.; Tong, C.; Fu, Q.; Xu, J.; Shi, S.; Xiao, Y.; *J. Pharm. Biomed. Anal*. **2019**, *170*, 153. [Crossref]
40. Torres, R.; Monache, F. D.; Bettolo, G. B. M.; *J. Nat. Prod*. **1979**, *42*, 430. [Crossref]
41. Johns, S. R.; Lamberton, K. A.; Sioumis, A. A.; *Aust. J. Chem*. **1968**, *21*, 1383. [Crossref]
42. Schmidt, J.; Raith, K.; Boettcher, C.; Zenk, M. H.; *Eur. J. Mass Spectrom*. **2005**, *11*, 325. [Crossref]
43. Hao, C.; Yang, W.; Dong, G.; Yu, Y.; Liu, Y.; Zhang, J.; Zhu, Y.; Wei, X.; Chen, S.; *Heliyon* **2023**, *9*, e16138. [Crossref]
44. Zhou, M.; Gong, X.; Ru, Q.; Xiong, Q. I.; Chen, L.; Si, Y.; Li, C.; *Neurotoxic. Res*. **2019**, *36*, 376. [Crossref]
45. Park, H.-J.; Baek, M.-Y.; Cho, J.-G.; Seo, K.-H.; Lee, G.-Y.; Moon, S.-J.; Baek, N.-I.; *J. Korean Soc. Appl. Biol. Chem.* **2011**, *54*, 345. [Crossref]
46. Hošt'álková, A.; Opletal, L., Kuneš, J.; Novák, Z.; Hrabinová, M.; Chlebek, J.; Cahlíková, L.; *Nat. Prod. Commun*. **2015**, *10*, 577. [Crossref]
47. Stévigny, C.; Jiwan, J. L. H.; Rozenberg, R.; de Hoffmann, E.; Quetin-Leclercq, J.; *Rapid Commun. Mass Spectrom*. **2004**, *18*, 523. [Crossref]
48. Oliveira, G. N. D. S. A.; Dutra, L. M.; Paz, W. H. P.; da Silva, F. M. A.; Costa, E. V.; da Silva Almeida, J. R. G.; *Biochem. Syst. Ecol.* **2021**, *97*, 104297. [Crossref]
49. Johns, S. R.; Lamberton, J. A.; Li, C. S.; Sioumis, A. A.; *Aust. J. Chem.* **1970**, *23*, 363. [Crossref]

50. Debourges, D.; Roblot, F.; Hocquemiller, R.; Cavé, A.; *J. Nat. Prod*. **1987**, *50*, 664. [Crossref]
51. Pérez, E.; Sáez, J.; Blair, S.; Franck, X.; Figadère, B.; *Lett. Org. Chem*. **2004**, *1*, 102. [Link] accessed in December 2023
52. Lai, Y. C.; Kuo, T. F.; Chen, C. K.; Tsai, H. J.; Lee, S. S.; *Drug Metab. Dis*. **2010**, *38*, 1714. [Crossref]
53. da Silva, F. M.; Bataglion, G. A.; de Almeida, R. A.; Heerdt, G.; Sousa, I. L.; da Silva Filho, F. A.; de Alencar, D. C.; Costa, E. V.; de Souza, A. D. L.; Pinheiro, M. L. B.; Morgon, N. H.; Koolen, H. H.; *Int. J. Mass Spectrom*. **2017**, *418*, 30. [Crossref]
54. Alias, A.; Hazni, H.; Mohd Jaafar, F.; Awang, K.; Ismail, N. H.; *Molecules* **2010**, *15*, 4583. [Crossref]
55. Brash, R. M.; Sneden, A. T.; *J. Nat. Prod*. **1983**, *46*, 437. [Crossref]
56. Barbosa-Filho, J.; Da-Cunha, E. V. L.; Cornélio, M. L.; Dias, C. S.; Gray, A. I.; *Phytochem*. **1997**, *44*, 959. [Crossref]
57. Lathifah, U. Z.; Rahim, R. A.; Sudrajat, H.; Khairi, S.; *J. Iran. Chem. Res.* **2010**, 59. [Link] accessed in December 2023
58. Mohamed, S. M.; Hassan, E. M.; Ibrahim, N. A.; *Nat. Prod. Res*. **2010**, *24*, 1395. [Crossref]
59. de Albuquerque, V. H. S. B.: *Estudo químico e biológico dos constituintes do Cerne de Abuta Refescens AUBL. (Menispermaceae)*; MSc. Dissertation, Universidade Federal do Amazonas, Manaus, 2004. [Link] accessed in December 2023
60. Boyom, F. F.; Fokou, P. V.; Yamthe, L. R.; Mfopa, A. N.; Kemgne, E. M.; Mbacham, W. F.; Tsamo, E.; Zollo, P. H.; Gut, J.; Rosenthal, P. J.; *J. Ethnopharmacol*. **2011**, *134*, 717. [Crossref]
61. Weniger, B.; Robledo, S.; Arango, G. J.; Deharo, E.; Aragon, R.; Munoz, V.; Callapa, J.; Lobstein, A.; Anton, R.; *J. Ethnopharmacol.* **2001**, *78*, 193. [Crossref]
62. Levrier, C.; Balastrier, M.; Beattie, K. D.; Carroll, A. R.; Martin, F.; Choomuenwai, V.; Davis, R. A.; *Phytochemistry* **2013**, *86*, 121. [Crossref]
63. Costa, J. O.; Barboza, R. S.; Valente, L. M. M.; Wolff, T.; Gomes, M.; Gallo, B.; Berrueta, L. A.; Guimarães-Andrade, I. P.; Gavino-Leopoldino, D.; Assunção-Miranda, I.; *J. Braz. Chem. Soc*. **2020**, *31*, 2104. [Crossref]
64. Arias, M. H.; Vallejo, G. A.; Garavito, G.; *Pharmacogn. Res.* **2021**, *13*, 4. [Crossref]
65. Shi, W.; Ting, L. M.; Kicska, G. A.; Lewandowicz, A.; Tyler, P. C.; Evans, G. B.; *J. Biol. Chem*. **2004**, *279*, 18103. [Crossref]
66. Swaminathan, P.; Saleena, L. M.; *Int. J. Comput. Biol. Drug Des*. **2019**, *12*, 1. [Crossref]
67. Graziose, R.; Rathinasabapathy, T.; Lategan, C.; Poulev, A.; Smith, P. J.; Grace, M.; Lila, M. A.; Raskin, I.; *J. Ethnopharmacol*. **2011**, *133*, 26. [Crossref]
68. Ferdous, A. J.; Islam, M. O.; Hasan, C. M.; Islam, S. N.; *Fitoterapia-milano* **1992**, *63*, 549. [Link] accessed in December 2023
69. Pyo, M. K.; Yun-Choi, H. S.; Hong, Y. J.; *Planta Med*. **2003**, *69*, 267. [Crossref]

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