In Silico Study of The Potential of Naturally Caffeoylquinic Acids From Lentinus Crinitus Basidiocarp With Zika Virus Inhibition Targets

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Abstract

The methanolic extract of the fungus Lentinus crinitus was submitted to biological assays, identification of the chemical composition by LC-MS, and in silico study by molecular docking with all identified compounds. The test against Artemis salina reached LD$_{50} > 1000$ μg/mL within 24h, and total mortality within 48 hours; the antioxidant test 62.4% inhibition in 1.0 mg/mL was obtained. Only 16 compounds were identified from the LC-MS analysis based on the comparison of reports already recorded in the literature. Most of the compounds identified here are described for the first time in the genus Lentinus. These results showed that the fungus is a producer of different classes of secondary metabolites biologically active. The results of the molecular docking simulation of the identified phytochemicals presented 1,13,4-di-O-Caffeoylquinic, as the leading promising candidate in the inhibition of the Zika virus.

Key Points

- Extract of the fungus Lentinus crinitus, the chemical composition by LC-MS.
- In silico study by molecular docking, evaluation of ecotoxicity, phytochemistry, and antioxidant
- 1,13,4-di-O-Caffeoylquinic as the candidate in the inhibition of the Zika virus.

Introduction

The term biodiversity, or biological diversity, describes the richness and variety of the natural world, plants, animals, and microorganisms that provide food, medicine, and much of the raw material consumed by man for decades (Purvis; Hector 2000).

Bacteria and fungi are the most studied among the most diverse microorganisms found in the Monera, Protista, and Fungi kingdoms. Fungi are microorganisms of complex nature that since antiquity have some of their species used as foods, due to the pleasant taste and their nutritional and medicinal properties, being also essential agents in various industrial processes such as obtaining enzymes, vitamins, antibiotic synthesis, extraction of polysaccharides, pigments, lipids, and glycolipids (Chang; Buswell, 1996). Previous studies have shown that these organisms produce many secondary metabolic scans with antitumor, antiviral, anti-inflammatory, antithrombotic, cytostatic, hypoglycemia, and antimicrobial activities (Ramos et al. 2010). Due to their industrial potential, fungi were previously seen as toxic are also used today as decomposers of these substances. Several studies have emerged, due to technological advances, thus demonstrating the interest of researchers focused on mycology, which is, therefore, the area of Biology destined to study fungi.

Fungi have been explored in medicine and other biotechnological processes involved in the production of enzymes and have presented great importance in the agricultural and ecological sectors, acting as a decomposer of plant residues and significantly degrading toxic substances symbiosis with plants (Nepomucena, 2010).

About their nature, fungi are eukaryotes, heterotrophic, achlorophilate, aerobic, microaerophilic, uni or pluricellular, with a cell wall usually composed of chitin and cellulose, in addition to other complex carbohydrates, with glycogen as a reserve substance, absorption nutrition, and may live as saprobes, parasites or symbionts with other organisms (Putzke; Putzke, 2004). They have the body formed by a tangle of filaments, called hyphae, and their set is called mycelium. Hyphae vary in diameter, wall thickness, and pigment location. They constitute an extensive and heterogeneous group found in any ecological niche. The number of fungal species spread worldwide is estimated at 1.5 million, of which only 74,000 species have been described. Excluding insects, fungi are the most numerous living microorganisms (Esposito; Azevedo, 2004).

This research arose from the need to conduct a study in the area of natural products to make a phytochemical analysis of the species of fungus that are born in the trees of the Baturité massif (hoses and the Indian denim tree), which are known as wooden ear, besides providing some information about the presence of secondary metabolites. The fungus Lentinus crinitus is a widespread species throughout Brazil and is often associated with discomfort trunks. They present centrally stipited,
infundibuliform basidoma and verrugous abhimenial surface to the strigose (Ballaminut, 2007). This work aims to set up assays to evaluate the functional capacity of the fungus *L. crinitus* regarding the antioxidant, ecotoxicological, and antimicrobial potential and make the phytochemical evaluation.

**Materials And Methods**

**Fungus collection and washing**

The fungus used was collected from a tree in the municipality of Redemption, in the Baturité massif in Ceará at the following coordinates: 4° 13′ 17" S, 38° 42′ 55" E and characterized and identified by image recognition in the Mushroom Identify - Automatic picture recognition application, and compared with the records available on the Virtual Herbarium of Flora and Fungi website with access allowed in the link: http://inct.florabrasil.net/herbario-virtual/.

**Fungus extraction**

After washing the fungus, 143.59 g of this material was weighed for extraction with methanol. The heavy sample was immersed in the solvent for 24 h. Then, the supernatant was vacuum filtered using a rotary evaporator to obtain the extract.

**Ecotoxicity test**

Ecotoxicity tests were designed to assess or predict toxic effects on biological systems and readjust the relative toxicity of substances (Forbes; Forbes, 2008). Toxicity assays can be used as the lethality assay with cotyledons microcrustacean developed to detect bioactive compounds in plant extracts. *Artemia salina* is a microcrustacean species of the order Anostraca, used as a bioindicator of toxicity (Meyer et al. 1982).

**Bioassay with Artemia salina**

A sodium chloride (NaCl) solution at a concentration of 30 g/L was prepared with pH 8. After the solution's preparation, a system was mounted inside a beaker of 1.75 L. The cup contained a screen with a diameter of 0.02 cm thick. Then, 1.50 L of the prepared saline solution was placed in the system. Later, about 50 mg of *Artemia salina* cysts were added on one side, with the care that they did not go to the other side of the container that contained only saline solution. For this, a 10 W LED lamp was positioned on the other side with just the saline solution to attract the organisms to the other side of the system, thus providing uniformity.

It is also boiling point that the temperature remained controlled around 28 to 30°C using a digital thermometer. The entire container was wrapped with aluminum foil to aid in the outbreak of cysts. The study's cysts for later use showed greater strength, uniformity of size, and attraction by light. This choice aims at homogenizing the physical conditions of test organisms. The incubation period for these organisms was around 48 hours. However, the presence of cysts was already evidenced in the first 24 hours.

**Ecotoxicity test against Artemia salina**

After the nauplius outbreak, those who overcame the dividing screen in the center of the beaker were removed from the system. These organisms overcame the dividing screen in the center of the cup opposite the side that was cultivated. They were captured with a Pasteur pipette and transferred to 12 test tubes to contain ten artemias in each box. The boxes contained 4 mL of the same prepared saline solution and 2 mL of the methanol extract in different concentrations (0.0 g/ml, 1.0 g/ml, 0.5 g/ml, 0.25 g/ml, 0.125 g/ml, and 0.0625 g/ml). The tests were performed in triplicate for each extract/compound concentration of this analysis. The counting of this biological assay was made by the first 24 and 48 hours after pipetting the larvae in the test tubes. In this study, living nauplius was considered, all those who presented movements inside the tube and were attracted to light, and dead, those at the boxes' bottom without any activities. A magnifying glass was used for better visualization and counting.
Antioxidant assay

Antioxidant activity was determined by capturing free radicals with the DPPH test. For this procedure's performance, Hegazi e El Hady's (2002) studies with some adaptations were used as references.

The methodology for antioxidant activity was based on the extinction measure of radical adsorption. This is because the DPPH has the purpura staining that is reduced, forming diphenylpicrilhydrazine of yellowish coloration. For this experiment, the methanol extract was methanol in several different concentrations (0.0 mg/ml, 1.0 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml, 0.0625 mg/ml). For this experiment, the fungus's methanol extract was diluted in 2 mL of methanol in several different concentrations, as represented in Table 2, except for the white sample, where only DPPH and Methanol were added. Only 2 mL of a DPPH solution was added to each concentration of 2 mL methanol extract of a solution of DPPH, except for the sample called a blank, where only the solvent was placed. After adding the DPPH in the absence of light, it waited 30 minutes for the solution to manifest. After that, the PG Instruments Ltd spectrophotometer at 520 nm was read. The measured absorbance of extract concentrations was converted into a percentage of antioxidant activity using equation 1.

\[
% \text{Inhibition} = \left( 1 - \frac{A_{sample}}{A_{blank}} \right) \times 100
\]  

(1)

Where: \( A_{sample} \) is the absorbance of the solution with DPPH.

\( A_{blank} \) is the absorbance of the DPPH solution without the sample.
Table 2
Data from the LC-MS spectrum and retention time (Rt) of the compounds detected in the methanolic extract of the fungus *Lentinus crinitus*, in positive ion mode [M-H]+.

| Peak | Rt (min) | [M-H]+ Observed | [M-H]+ Calculated | Empirical Formula | ppm (error) | Phytochemical Name | References |
|------|----------|------------------|-------------------|------------------|-------------|---------------------|------------|
| 1    | 0.94     | 330.1699         | 330.1710          | C_{19}H_{25}NO_{4} | -3.3        | Erythratidine       | (Amer; Shamma; Freyer, 1991; Feitosa et al. 2012) |
| 2    | 1.0      | 314.1741         | 314.175           | C_{19}H_{24}NO_{3} | -4.8        | Erisotrina          | (Amer; Shamma; Freyer, 1991) |
| 3    | 2.50     | 325.1070         | 325.1076          | C_{19}H_{16}O_{5}  | -1.8        | Eucalyptin          | (Zapesochnaya; Sokol'skaya, 1984) |
| 4    | 3.92     | 457.1365         | 457.1346          | C_{20}H_{25}O_{12} | 4.2         | 15-demethylplumeride | (Hassan et al. 2008) |
| 5    | 6.02     | 181.0482         | 181.0501          | C_{9}H_{8}O_{4}    | -1.1        | Caffeic acid        | (Guo et al. 2008) |
| 6    | 7.32     | 317.0312         | 317.0297          | C_{15}H_{9}O_{8}   | 4.7         | Gossypetin          | (Dudek; Warskulat; Schneider, 2016) |
| 7    | 7.77     | 219.1740         | 219.1749          | C_{15}H_{23}O      | -4.1        | (+)- Nookatone      | (Wu, 2009) |
| 8    | 8.72     | 481.1358         | 481.1346          | C_{22}H_{24}O_{12} | 2.5         | Noidesol            | (Shimokawa et al. 2010) |
| 9    | 9.95     | 403.1411         | 403.1393          | C_{21}H_{23}O_{8}  | 4.5         | Nobletin            | (Wang et al. 2007) |
| 10   | 10.75    | 921.7843         | 921.7860          | C_{62}H_{105}O_{7} | -1.8        | unidentified        |            |
| 11   | 12.20    | 340.2855         | 340.2852          | C_{20}H_{38}NO_{3} | 0.9         | unidentified        |            |
| 12   | 13.66    | 365.1371         | 365.1389          | C_{22}H_{21}O_{5}  | -4.9        | Asperterone         | (Nuclear et al. 2010) |
| 13   | 15.07    | 751.4422         | 751.4421          | C_{44}H_{63}O_{10} | 0.1         | unidentified        | -          |
| 14   | 15.41    | 425.2108         | 425.2117          | C_{29}H_{29}O_{3}  | -2.1        | unidentified        | -          |
The reduction of the DPPH radical is followed by monitoring the decrease of its absorbance to a characteristic wavelength during the reaction. The graphs were plotted using the Origin version 9.0 program. The value of \( IC_{50} \), inhibiting concentration (CI), of the fungus extract capable of reducing 50% of the DPPH radical, was obtained by replacing in the equation of the line \( y = ax + b \), where \( y = 50 \) by the least-squares method for the graph obtained. From the % inhibition values, the axes of the (coordinates) and extract concentrations were assembled, and the (abscissa) axes were made.

**Chromatographic analysis**

The analysis was performed in an Acquity UPLC (Waters) system, coupled to a Quadrupole/Flight Time (QtoF, Waters) system belonging to the Brazilian Agricultural Research Company (EMBRAPA). Chromatographic runs were performed on a Waters Acquity UPLC BEH column (150 x 2.1 mm, 1.7 a), the fixed temperature of 40 °C, mobile phases, water with 0.1% formic acid (A), and acetonitrile with 0.1% formic acid (B), gradient ranging from 2–95% B (15 min), the flow of 0.4 mL/min and injection volume of 5 ul.

**High-Resolution Mass Conditions - XEVO-QToF**

The ESI- model was acquired in the range of 110 -1180 Da, fixed source temperature at 120 °C, desolvation temperature 350 °C, desolation gas flow of 500 L/h, extraction cone of 0.5 V, the capillary voltage of 2.6 kV. The ESI+ mode was acquired in the range of 110 - 1180 Da, fixed source temperature of 120 °C, desolation temperature 350 °C, desolation gas flow of 500 L/h, and capillary voltage of 3.2 kV. Leucine encephalin was used as a lock mass. The acquisition mode was MSE. The instrument was controlled by masslynx 4.1 software (Waters Corporation).

**Computational simulation Procedures**

**Computational procedures**

The simulation was performed using the Autodock Vina code (Trott; Oslon, 2009), where it was also used to optimize and calculate Autodotck Tools visualization (Morris et al. 2009), Avogadro (Hanwell et al. 2012), and the Discovery Studio viewer (Biovia, 2015).

**Obtaining protein structure**

Obtaining the protein transferase of zika virus was made in the Protein Data Bank (https://www.rcsb.org/structure/5M5B), repository, where it was deposited with the code 5M5B, which was generated from X-ray diffraction, with a resolution of 2.01 Å, R-Value Free: 0.191, with unit cell a = 37.55 Å, b = 64.14 Å, c = 72.04 Å, \( \alpha = 113.05^\circ \), \( \beta = 92^\circ \), \( \gamma = 90.00^\circ \), being classified as transferase, expressed in *Escherichia coli*, BL21 (Coutard et al. 2017).

**Optimization of Structures**
For structural optimization and electronic characterization of the molecule, all optimization calculations were performed in the Software Avogadro®, with a field MFF94. Avogadro is software that allows theoretical performance calculations (optimization of geometry, energy, and properties) at the level of theory MM, EHT, AM1 / PM3, and MNDO / ZINDO (Hanwell et al. 2012; De Souza et al. 2021).

**Molecular Docking**

Molecular fixation of ligands to Zika virus transferase receptor was performed using the AutoDock Tools graphical interface (4.2.6) that performs AutoDock Vina (1.1.2).

**Results**

**Antioxidant activity**

A statistical treatment with all the data collected about the antioxidant activity to verify the percentage of inhibition of DPPH and in which concentration was higher the sequestration of free radical DPPH. Table 1 is below, which contains all data on antioxidant activity.

| Concentration (mg/mL) | Conc. (ppm) | % Inhib. | Abs.  | Abs.  | Abs.  |
|-----------------------|-------------|----------|-------|-------|-------|
|                       |             |          |       |       |       |
| 1.0                   | 1.000       | 62.3     | 0.235 | 0.235 | 0.236 |
| 0.5                   | 500.0       | 27.2     | 0.454 | 0.455 | 0.456 |
| 0.25                  | 250.0       | 19.1     | 0.504 | 0.506 | 0.506 |
| 0.125                 | 125.0       | 9.9      | 0.562 | 0.563 | 0.564 |
| 0.0625                | 32.5        | 3.7      | 0.601 | 0.602 | 0.603 |
| DPPH                  |             |          | 0.625 |       |       |

The IC$_{50}$ value was calculated using the values obtained after linear regression, with the application of the straight equation ($y = ax + b$) originated from the Fig. 1, which already shows the concentration curve of the fungus's methane extract ($\mu$g/mL) versus the percentage of DPPH Inhibition (% Inhibition).

The results of the antioxidant potential of five mushroom species identified as *Pleurotus sp.*, *Hygrocybe sp.*, *Hygrophorus sp.*, Schizophyllum commune, and Polyporus tenuiculust. From each mushroom, two extracts were obtained, one with petroleum ether and the other with methanol.

**Chromatographic identification of chemical constituents in the methanol extract**

The high-resolution LC-MS significantly improves the possibility of obtaining valuable information from different chromatographic profiles in an extract (Zou et al. 2014).

Liquid chromatography coupled with mass spectrometry (LC-MS/MS) was successfully applied in this study to identify the chemical constituents and their secondary metabolites present in the methanol extract of the fungus *Lentinus crinitus*. It was possible to identify a total of 16 compounds in positive ion modes and negative ion modes. Tables 2 and 3 show the
compounds identified by the attempt method, compared with the literature records based mainly on their molecular ions, retention time, and presented fragmentations.

Table 3
Data from the LC-MS spectrum and retention time (Rt) of the compounds detected in the methanolic extract of the fungus *Lentinus crinitus*, in negative ion mode [M-H]-.

| Peak | Rt  | [M-H]^- | [M-H]^- | Product Ions (MS/MS) | Empirical Formula | Ppm (error) | Phytochemical Name | References |
|------|-----|----------|----------|----------------------|------------------|-------------|-------------------|------------|
| 1    | 0.51| 325.1866 | 325.1862 | 311, 325, 297, 183, 83 | C_{14}H_{29}O_{8} | 1.2         | unidentified      | -         |
| 2    | 1.39| 191.0165 | 191.0133 |                      | C_{13}H_{3}O_{2} | 1.6         | unidentified      | -         |
| 3    | 1.40| 515.1248 | 515.1190 | 353.0876, 173.0453, 179.03445, 191.0559 | C_{25}H_{29}O_{12} | 2.1         | 3,4-di-O-Caffeoylquinic acid | (Ooi et al. 2011) |
| 4    | 2.50| 325.1070 | 325.1076 | 325.0946, 124.0075, 223.0419 | C_{19}H_{18}O_{5} | -1.8        | Eucalyptin        | (Zapeschnaya; Sokol'skaya, 1984) |
| 5    | 6.08| 197.0432 | 197.0450 | 197.0398; 167.0303; 153.0456 | C_{9}H_{9}O_{5} | -2.8        | Siringic acid     | (Hoffmann; Linuma; Herrmann, 2007) |
| 6    | 7.35| 167.0367 | 167.0344 | 131.0181; 167.0381; 116.9339 | C_{8}H_{7}O_{4} | 4.8         | Vanillic acid     | (Hoffmann; Linuma; Herrmann, 2007) |
| 7    | 9.31| 329.2309 | 329.2328 | 329.2355, 199.1314, 183.0111 | C_{18}H_{33}O_{5} | -5.8        | Pinelic acid      | (Sunnam; Prasad, 2013) |
| 8    | 12.64| 315.2512 | 315.2535 | 315.2529, 183.0113 | C_{18}H_{35}O_{4} | -7.3        | 9,10-Dihydroxystearate acid | (Koay et al. 2011) |
| 8    | 18.06| 253.2530 | 253.2531 | 183.0066, 253.2543, 197.0254 | C_{17}H_{33}O | -0.4        | unidentified      | -         |
| 9    | 21.59| 325.1795 | 325.1804 | 325.1822, 183.0089, 311.1673, 197.0241 | C_{21}H_{29}O_{3} | 0.3         | unidentified      | -         |

**Description of peaks displayed on chromatogram in positive mode**

Peak 1, molecular ion, with mass/load ratio (m/z) equal to 330.1699 was found in the methanol extract *Lentinus crinitus*, with an Rt = 0.94 and presented the fragments (183; 198; 297; 223), which corresponded to the compound previously identified as Erythratidine (Amer; Shamma; Freyer, 1991; Feitosa et al. 2012). Peak 2 presented at m/z 314.1741 with an Rt = 1.00 and gave the fragments (313.37, 298.32, 283.28), corresponding to the previously identified compound Erysotrine. Both belong to the class of Alkaloids, unpublished in the genus *Lentinus crinitus*. Peak 3, molecular ion, with mass/load ratio equal to 325.1070. It was found in the methanol extract *Lentinus crinitus*, with an Rt = 2.5, and presented the fragments (138.0598, 204.0835, 325.0946, 124.0075, 223.0419), which corresponded to the compound previously identified and yet reported in the literature as Eucalyptin. Belonging to the flavonoid class, a phenolic compound unheard of in the genus *Lentinus crinitus*. 
Several authors have reported phenolic compounds in several edible fungal extracts and different areas in Portugal, Spain, and Finland (Kim et al. 2008; Ribeiro et al. 2008; Jayakumar; Thomas; Geraldine, 2009; Palacios et al. 2011). Peak 4, with m/z 457.1365, was found in the extract with an Rt = 3.92 and presented the fragments (204.0932, 187.1129, 136.0653), which corresponded to the compound previously as a new alkaloid monoterpene called 15-demethylplumeride. Peak 5 with molecule ion m/z 181.0482 was found in the methanolic extract Lentinus crinitus, with an Rt = 6.02, and presented the fragments (151.0436; 181.0499; 133.0827), which corresponded to caffeic acid, identified early (Guo et al. 2008). An unprecedented compound for species, as there are no reports of these compounds' presence in the literature. Caffeic acid is an acid derived from catechol and can be found generally in several plant species, such as teas, coffee, and elderflower (Meinhart et al. 2017). As for peak 6, the molecular ion with m/z = 317.0312 was found in the extract, with Rt = 7.32, with the fragments (319.12; 219.17; 133.09, 220.17), which corresponded with gossypetin reported in the literature, is a hexahydroxyflavone with hydroxyl groups placed in positions 3-, 3'-, 4', 5-7 and 8- and it works like a vegetable metabolite. According to the literature, a flavonoid in the genus Lentinus crinitus has not yet been identified. However, it is known that phenolic compounds are generated as secondary metabolites in plants and fungi. They are considered one of the most influential groups associated with antioxidant power, having already described their ability to melt metals and inhibit lipoxygenase and free radicals (Decker, 2009). Peak 7, whose molecular ion obtained is m/z = 219.1740, was found in the extract with an Rt = 7.77, presenting the various fragmentations (121, 181, 147, 203, 200) that allowed identifying the compound belonging to the class of sesquiterpenoid named (+) - Nootkatone. The presence of sesquiterpenes in the genus Lentinus was first reported by literature, (Wu, 2009), which was able to identify a total of 19 sesquiterpene compounds in several Lentinus species. Basidiomycetes are a rich source of terpenoids forming mushrooms (Schmidt-Dannert, 2014). Sesquiterpenoids are important secondary metabolites and have several pharmaceutical and nutraceutical properties. In particular, the upper basidiomycetes have a versatile biosynthetic repertoire for these bioactive compounds (Lee et al. 2020). Peak 8, a molecular ion with an m/z ratio equal to 481.1358, was found in the methanol extract Lentinus crinitus, with an Rt = 8.72 and presented the fragments (293.0456, 151.0406, 133.0764), which corresponded to the compound previously identified and reported in the literature as Noidesol A, which is a dihydrophenol-C-glycosides, which was isolated from the bark of a woody plant of the genus Gnetum gnemonoides (Shimokawa et al. 2010). Peak 9, a molecular ion with mass/load ratio equal to 403.1411, was found in this study with Rt = 9.52, and presented the fragments (402.13, 403.13), the search in the literature corresponded to Nobiletin, [systematic name: 2-(3,4-dimethoxyphenyl) -5,6,7,8-tetramethoxy-4H-chromen-4-one (Meinhart et al. 2017). As for peak 6, the molecular ion with m/z 181.0482 was found in the methanolic extract Lentinus crinitus, in negative ion mode with an Rt = 2.50 and presented the fragments (138.0598, 204.0835, 325.0946, 124.0075, 223.0419), which corresponded to the previously identified compound, already in positive mode, and reported in the literature as Eucalyptin, belonging to the flavonoid class. Peak 5, a molecular ion with m/z equal to 325.1070, was found in the methanol extract Lentinus crinitus, in negative ion mode with an Rt = 6.08 and presented the fragments (197.0398; 167.0303; 153.0456), which corresponded to Siringic Acid. Siringic acid plays a role in the communication between plants and soil microorganisms through changes in the microbial communities of the soil rhizosphere and inhibition of cucumber seedlings’ growth. It has antioxidant, antimicrobial, anti-inflammatory, and anti-
endotoxic activities (Srinivasulu et al. 2018). Vanillic acid is a phenolic compound used with an extended stay in the industry as a flavoring and sometimes as a food preservative, and can be found in cereals, whole grains, fruits, herbs, green tea, juices, beers and has antioxidant, hepatoprotective, cardioprotective and antiapoptotic activities (Marakov; Uchuskin; Trushkov, 2018). Peak 7, a molecular ion with a mass/load ratio equal to 167.0367, was found in the methanol extract Lentinus crinitus, in negative mode with an Rt = 7.35 and presented the fragments (131.0181; 167.0381; 116.9339), which corresponded to vanillic acid (Hoffmann; Linuma; Herrmann, 2007). Vanillic acid is a phenolic compound used with an extended stay in the industry as a flavoring and sometimes as a food preservative, and can be found in cereals, whole grains, green tea, juices, beers and has antioxidant, hepatoprotective, cardioprotective and antiapoptotic activities (Almeida; Cavalcante; Vicentini, 2016). Peak 8, a molecular ion with mass/load ratio equal to 315.2512, was found in the methanolic extract Lentinus crinitus, in negative mode with an Rt = 12.64 and presented the fragments (315.2529, 183.0113), which corresponded to a fatty acid developed in Malaysia named 9,10-Dihydroxystearate acid (Koay et al. 2011).

Figure 4 and 5 describe the possible structures of the identified compounds.

**Ecotoxicity test against Artemia salina**

The mortality results of Artemia salina larvae obtained with the methanol extract of the fungus Lentinus crinitus were examined after the count of 24 and 48 hours and are represented in Table 4. Depending on the toxicity analysis, it became possible to determine the percentage of dead Artemia salina nauplius and thus determine LD$_{50}$, the concentration necessary to kill 50% of the sample population (Artemia salina) the stratum.

| Concentration | Tube 1 | Tube 2 | Average 24h | Tube 1 | Tube 2 | Average 48h |
|---------------|--------|--------|-------------|--------|--------|-------------|
| 1.000 mg/mL   | 3.0    | 2.0    | 2.5         | 10.0   | 10.0   | 10.0        |
| 0.500 mg/mL   | 6.0    | 5.0    | 5.5         | 10.0   | 10.0   | 10.0        |
| 0.250 mg/mL   | 4.0    | 4.0    | 4.5         | 10.0   | 10.0   | 10.0        |
| 0.125 mg/mL   | 1.0    | 1.0    | 5.0         | 9.0    | 10.0   | 9.0         |
| 0.062 mg/mL   | 5.0    | 5.0    | 4.5         | 10.0   | 10.0   | 10.0        |
| 0.031 mg/mL   | 5.0    | 3.0    | 4.0         | 10.0   | 10.0   | 10.0        |
| Blank         | 0.0    | 0.0    | 0.0         | 0.0    | 0.0    | 0.0         |

The criterion of classification of the methanol extract of the fungus extract against Artemia salina used in this work was based on the values of LD$_{50}$ established by literature (Meyer et al. 1982) using the evaluation criterion in which the sample is considered toxic or active those with LD$_{50}$ < 1000 µg/mL and nontoxic or inactive samples with LD$_{50}$ > 1000µg/mL. Depending on the tabled data, the construction of a graph that more clearly exposes the results obtained was performed, making it possible to graphically analyze all the data obtained after the calculations were performed. The Figure S1 explains the data mentioned above in Table 4 about 24 hours because the same extract presented total mortality during 48 hours.

**Molecular Docking with Compounds Identified Against Zika Virus Transferase**

This study phase performed docking calculations for the 16 compounds newly identified by LC-MS/MS. The biological target selected was 5M5B (Zika virus transferase). The active site in which the molecules were embedded was the same as the native SAM (S-Adenosilmethionine) alloy, as shown in Table 5.
Table 5
SAM (S-Adenosilmethionine) native alloy grid used for Docking.

| Parameter | Value     |
|-----------|-----------|
| center_x  | 8.607370  |
| center_y  | 43.187481 |
| center_z  | 86.285630 |
| size_x    | 40.0      |
| size_y    | 40.0      |
| size_z    | 40.0      |

Visualization of Receptor-Ligand Complex Interactions

The Discovery Studio, was used to visualize the interactions of the receptor-ligand. The choice was made due to the ease of handling the software and its optimized graphics that facilitate understanding the results more straightforwardly, as shown in Figure S3a-S3b (Biovia, 2015). In Figure S3a, it is possible to observe the result obtained orange to SAM in the co-crystallized form and in blue the SAM obtained by redocking with affinity energy equal to -7.3 Kcal/mol and the deviation in RMSD in redocking by binding at the same site. The affinity energy linker was equal to -7.3 Kcal/mol and more minor variations in RMSD with 1.856 Å. Furthermore, Figure S3b shows the Docking of the compound 13,4-di-O-Caffeoylquinic acid (red) with the protein anchoring in the same region of the SAM (orange), with energy value -8.8 Kcal/mol and the deviation in RMSD equal to 1.872 Å and its affinity Ki (µM) = 0.689. The result is satisfactory since the ligands were coupled at the same active site of the target protein.

From Figure S4a and S4b, it is possible to verify the compounds' interactions with the central amino acid residues, highlighting Asp152, Trp93, Gly92, Gly64, for 3,4-di-O-Caffeoylquinic acid (compound 1), and SAM. 3D visualization facilitates understanding and visibility of the amino acids present. Figure S5 more specifies the ratio by highlighting the distances separately for the compounds.

Figure S5 shows the main interactions between protein residues and compound 1,4-di-O-Caffeoylquinic acid. The outstanding approximations were Van Der Waals' interactions with his 116, Gly 112, Ser 65, Gly 91, Lys 188, Val 136, Phe 139, Thr 110, and Arg 90; Other interactions were observed, such as two pi-alkyl interactions, with Ile 153 (4.84 Å and 5.74 Å) residues, and two conventional hydrogen bonds between Asp 137 (3.64 Å) and Val 138 (3.62 Å).

Discussion

Antioxidant activity

The extract presented plausible inhibition percentages. It gave antioxidant action of 62.3% at the concentration of 1000 µg/mL. At the concentrations analyzed, the same extract with the maximum absorbance value of 0.602 in the concentration was 32.5 µg/mL. According to the description of Fig. 1, it is perceived that the higher the concentration, the higher the percentage of inhibition. It agreed with a study, which states that the higher the DPPH intake by the sample, the higher its antioxidant activity (Nascimento et al. 2011). Therefore, it is verified similarly that the higher the sample's concentration, the lower the absorbance, and the higher the DPPH consumption.

Analysis of some edible wild mushrooms' nutritional and antioxidant potential capacity of the genus Lentinus crinitus using DPPH elimination assay methanolic extracts determined that the mushrooms presented significant antioxidant activities (Ao; Deb, 2019). Among the ten species studied, Lentinus tigrinus given about (47.5 µg/ml, IC50) exhibited the largest
sequester of DPPH radicals, followed by *Lentinus squarrosulus* (82.6 µg/ml, IC50). The IC50 value was calculated to study the inhibition capacity of mushrooms against DPPH radicals.

The authors concluded that the mushroom species showed appreciable DPPH radical sequestration activity, the oil ether extract of *Pleurotus* sp., which showed the highest % inhibition (83.04 %) concentration of 20 mg/mL (20,000 µg/mL). This ability to kidnap the free radical DPPH was twice that of *Hygrocybe* sp (Chye; Wong; Lee, 2008).

When evaluating the antioxidant potential of the fungus *Tyllopiillus ballilloui*, found that it proved to be a good source of compounds with antioxidant activity. Other studies have shown promising results when evaluating the antioxidant activity of compounds produced by fungi, such as *Penicillium citrinum*, the endophilic fungi *Viscumalbum L.* and *Penicillium sp.* However, few results were found in the reviewed literature on the antioxidant activity of the methanol extract of the fungus *Lentinus crinitus* (Lima, 2009).

The analysis of the methanol extracts of various mushroom species presented significant antioxidant activity in vitro. The authors suggest that mushrooms can be a natural source of antioxidants, as a food supplement or in the pharmaceutical industry, and phenolic compounds are the main ones responsible for the antioxidant activity of edible mushrooms (Elmastas et al. 2007).

Compared to more potent natural antioxidants, the results obtained show considerably high activity for the fraction analyzed.

The present study suggests that *Lentinus crinitus* fungi can aid oxidative damage in cells induced by oxygen radicals and effectively exploit antioxidant potential in the medical-pharmaceutical area.

**Chromatographic identification of chemical constituents in the methanol extract**

Chromatography is a fundamental technique used to quantify various fungal species’ chemical constituents and has been used by several authors in several studies. Researchers used high-efficiency reverse-phase liquid chromatography with a diode arrangement detector to analyze phenolic acids present in aqueous and methanolic extracts of mushrooms (Puttaraju et al. 2006). While also analyzed phenolic compounds of mushrooms with HPLC system and diode arrangement detector. Some phenolic acids were identified in 19 mushrooms from Poland, using high-efficiency reverse-phase liquid chromatography and electrospray ionization mass spectrometry (LC-ESI-MS/MS) (Nowacka et al. 2014).

The chromatogram showed the equivalent of 21 peaks in positive mode and nine peaks in negative mode. Only ten peaks were identified in positive mode and six in negative mode, highlighting the existence of distinct chemical classes present in the extract, such as Alkaloids, flavonoids, sesquiterpenes, phenolic acids, and other types of compounds. The presence of flavonoid and phenolic acids justifies the high antioxidant content that the extract presented.

Our analysis results indicate that the extract’s chemical profile is satisfactory because unpublished compounds were identified using this technique, not yet described in the literature to date, as shown in Fig. 2 and 3 below, based on their peaks and retention times.

**Description of peaks displayed on chromatogram in positive mode**

*Ecotoxicity test against Artemia salina*

According to Figure S1 behavior, it was possible to conclude that the mortality of *Artemia salina* in 24 hours increases linearly as the concentration increases. Similarly, it was also perceived that the lower the concentration of the fungus extract, the lower the mortality of larvae. Thus, the lethal dose (LD$_{50}$) was calculated based on the line equation obtained by linear regression, considering the correlation of the logarithm of concentrations and the corresponding percentage of mortality. At
the value of y (coordinates) is attributed to half of the maximum possible deaths (n/2), the result of x obtained (abscissas) the antilogarithm is applied, resulting in the final value of the DL$_{50}$ (Rajeh et al. 2012).

The calculated LD$_{50}$ was equal to 2,747.286 µg/mL by constructing the linear regression, classified as a non-toxic sample. The absence of cytotoxicity against Artemia salina indicates that the part of the evaluated extract can be well tolerated given the biological system. The finding of potential toxicological signals the need for further studies such as in vitro tests to clarify the aspects of toxicity. Although cytotoxicity represents a concern regarding the safety of use, recent studies address cytotoxic plants’ antitumor potential (Pereira et al. 2015).

*Molecular Docking with Compounds Identified Against Zika Virus Transferase*

Molecular docking was successfully applied in this study using the AutoDock Vina software (Trott; Oslon, 2009). The compounds used as binders went through the entire three-dimensional space of the grid until finding the most favorable positions; at the end of the calculation, RMSD values were obtained for the poses where the ligands were coupled that are described in Table S1, with their affinity energies and the inhibition constant that was determined by the docking energy. As the docking works in the validation of the affinity of the luminant present in a given active site found in the receiver of interest, to affect the confirmation of the anchoring in this study, affinity energies lower than the redocking value of the SAM, of -7.3 Kcal/mol and RMSD deviations more minor than two angstroms (Å) were taken into account.

Table S1 shows the three ligands that presented lower dating energy and $K_i$ than the native ligand SAM, 3,4-di-O-Caffeoylquinic acid, Gossypetin, and Asperterone. Thus, it was necessary to draw Figure S2 to explain the relationship between RMSD and $K_i$ better. The RMSD and the inhibition constant provide information on the stability of the receptor-ligand complex formed. The smaller $K_i$, the greater the strength of the complex and, consequently, the greater the efficacy of the ligand in inhibiting the receptor (Junior; Arlan, 2019).

**Declarations**

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**COMPLIANCE WITH ETHICAL STANDARDS**

**CONFLICT OF INTEREST**

The authors declare that they have no conflict interests.

**ETHICAL APPROVAL**

This article does not contain any studies done with human or animal participants performed by any authors.

**CONSENT TO PARTICIPATE**

All the authors have given consent for the participation of the manuscript.

**CONSENT TO PUBLISH**

All authors accept that the copyright of the manuscript will be transferred to the journal upon acceptance for publication.

**AUTHORS CONTRIBUTIONS**
DFAM, SNB, CRP, CEDL, RPRV, DSJSN: Research, methodology, data collection, and writing

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COMPETING INTERESTS

The authors declare no competing interests.

AVAILABILITY OF DATA AND MATERIALS

The datasets in this work are available from the corresponding author upon reasonable request.

References

1. Almeida I, Cavalcante F, Vicentini V (2016) Different responses of vanillic acid, a phenolic compound, in htc cells: Cytotoxicity, antiproliferative activity, and protection from DNA-induced damage. Genet. Mol. Res 15:4

2. Amer ME, Shamma M, Freyer AJ (1991) The Tetracyclic *Erythrina Alkaloids*. J Nat Prod 54:329–363

3. Ao T, Deb CR (2019). Nutritional and antioxidant potential of some wild edible mushrooms of Nagaland, India. J. Food Sci. Technol 56:1084-1089

4. Ballaminut N, (2007) Caracterização fisiológica do inóculo de *Lentinus crinitus* (L .) Fr. CCB274 empregado em biorremediação de solo

5. Biovia (2015) Dassault Systemes BIOVIA, Discovery Studio Modelling Environment, Release 4.5. Accelrys Softw. Inc.

6. Chang ST, Buswell JA (1996) Mushroom nutriceuticals. World J. Microbiol. Biotechnol 12:473-476

7. Chye FY, Wong JY, Lee JS (2008). Nutritional quality and antioxidant activity of selected edible wild mushrooms. Food Sci Technol Int 14:375-384

8. Coutard B, Barral K, Lichière J, Selisko B, Martin B, Aouadi W, Lombardia MO, Debart F, Vasseaur JJ, Guillemot JC, Canard B, Decroly, E. (2017). Zika virus methyltransferase: structure and functions for drug design perspectives. J. Virol 91:e02202-16

9. De Souza LD, Silva BV, Araujo Neto WN, Rezende MJC (2021). Digital technologies in chemistry teaching: A brief review of the available categories and tools. Rev. Virtual de Quimica, 713-746

10. Decker A (2009) Phenolics: Prooxidants or Antioxidants? Nutr Rev 55:396–398

11. Dudek B, Warskulat A-C, Schneider B (2016) The Occurrence of Flavonoids and Related Compounds in Flower Sections of Papaver nudicaule. Plants 5:28.

12. Elmastas M, Isildak O, Turkekul I, Temur N (2007) Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. J Food Compos Anal 20:337–345

13. Esposito E, Azevedo JD (2004). Fungos: uma introdução à biologia, bioquímica e biotecnologia. Caxias do Sul: Educys, 510.

14. Feitosa LGP, Guaratini T, Lopes JLC, Lopes NP, Bizaro AC, Da Silva DB (2012) Aplicação de espectrometria de massas com ionização por elétron na análise de alcaloides do mulungu. Quim Nova 35:2177–2180
15. Forbes VE, Forbes TL (1994) Ecotoxicology in Theory and Practice, 4a. Chapman & Hall, Londres
16. Guo X, Chen X, Li L, Shen Z, Wang X, Zheng P, Duan F, Ma Y, Bi K (2008) LC–MS determination and pharmacokinetic study of six phenolic components in rat plasma after taking traditional Chinese medicinal-preparation: Guanxinning lyophilized powder for injection. J Chromatogr B 873:51–58
17. Hanwell MD, Curtis DE, Lonie DC, Vandermeersch T, Zurek E, Hutchison GR (2012). Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. J. Cheminformatics 4, 17
18. Hassan EM, Shahat AA, Ibrahim NA, Vlietinck AJ, Apers S, Pieters L (2008) A new monoterpenyl alkaloid and other constituents of Plumeria acutifolia. Planta Med 74: 1749-1750.
19. Hegazi AG, Abd El Hady FK (2002). Egyptian propolis: 3. Antioxidant, antimicrobial activities and chemical composition of propolis from reclaimed lands. Z. Naturforsch. C, 57:395-402
20. Hoffmann D, Linuma Y, Herrmann H (2007) Development of a method for fast analysis of phenolic molecular markers in biomass burning particles using high performance liquid chromatography/atmospheric pressure chemical ionisation mass spectrometry. J Chromatogr A 1143:168–175
21. Hwang SL, Shih PH, Yen GC (2015) Citrus Flavonoids and Effects in Dementia and Age-Related Cognitive Decline Diet and Nutrition in Dementia and Cognitive Decline pp:869-878
22. Jayakumar T, Thomas PA, Geraldine P (2009) In-vitro antioxidant activities of an ethanolic extract of the oyster mushroom, Pleurotus ostreatus. Innov Food Sci Emerg Technol 10:228–234
23. Júnior EGSS, Arlan ADSG (2019) Estudo Por Docagem Molecular De Inibidores Da Enzima: GSK-3β Uma Proposta Para O Tratamento Do Transtorno Bipolar. Rev Ifes Ciência 5:243–256
24. Kim M-Y, Seguin P, Ahn J-K, Kim J-J, Chun S-C, Kim E-H, Seo S-H, Kang E-Y, Kim S-L, Park Y-J, Ro H-M, Chung I-M (2008) Phenolic Compound Concentration and Antioxidant Activities of Edible and Medicinal Mushrooms from Korea. J Agric Food Chem 56:7265–7270
25. Koay GF, Chuah TG, Zainal-Abidin S, Ahmad S, Choong TS (2011) Development, characterization and commercial application of palm based dihydroxystearic acid and its derivatives: an overview. J Oleo Sci 60:237-265
26. Lee J, Hilgers F, Loeschke A, Jaeger KE, Feldbrügge M (2020) Ustilago maydis serves as a novel production host for the synthesis of plant and fungal sesquiterpenoids. Front. microbiol 11:1655
27. Lima ATM (2009). Avaliação do potencial antioxidante e antiinflamatório de galactomanana do fungo Tylopilus balilouii (Master's thesis, Universidade Federal do Rio Grande do Norte)
28. Makarov AS, Uchuskin MG, Trushkov, IV (2018) Furan oxidation reactions in the total synthesis of natural products. Synthesis 50:3059-3086
29. Meinhart AD, Damin FM, Caldeirão L, Da Silveira TFF, Filho JT, Godoboy HT (2017) Chlorogenic acid isomer contents in 100 plants commercialized in Brazil. Rev Ifes Ciência 99:522–530
30. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DJ, McLaughlin JL (1982) Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 45:31-34.
31. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK,Goodsell DS, Olson AJ (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J. Comput. Chem 30:2785-2791
32. Nascimento JC, Lage LFO, Camargos CRD, Amaral JC, Costa LM, Sousa AD, Oliveira FQ (2011). Determinação da atividade antioxidante pelo método DPPH e doseamento de flavonóides totais em extratos de folhas da Bauhinia variegata L. Rev.Bras. Farm 92:327-332
33. Nepomucena R, Maria P (2010) Avaliação do Potencial Microbiano de Crescimento e Secreção de lacase do Fungo Amazônico Lentinus crinitus (L.ex Fr.) Fr. Universidade do Estado do Amazonas
34. Noguchi S, Atsumi H, Iwao Y, Kan T, Itai S (2016) Nobiletin: a citrus flavonoid displaying potent physiological activity. Acta Crystallogr. C 72:124-127
35. Nowacka N, Nowak R, Drozd M, Olech M, Los R, Malm A (2014) Analysis of phenolic constituents, antiradical and antimicrobial activity of edible mushrooms growing wild in Poland. LWT - Food Sci Technol 59:689–694
36. Nuclear P, Sommit D, Boonyuen N, Pudhom K (2010) Butenolide and furandione from an endophytic *Aspergillus terreus*. Chem. Pharm. Bull. 58(9), 1221-1223
37. Ooi KL, Muhammad TST, Tan ML, Sulaiman SF (2011) Cytotoxic, apoptotic and anti-α-glucosidase activities of 3,4-di-O-caffeoyl quinic acid, an antioxidant isolated from the polyphenolic-rich extract of *Elephantopus mollis Kunth*. J Ethnopharmacol 135:685–695
38. Palacios I, Lozano M, Moro C, D’Arrigo M, Rostagno MA, Martínez JA, García-Lafuente A, Guillamón E, Villares A (2011) Antioxidant properties of phenolic compounds occurring in edible mushrooms. Food Chem 128:674-678
39. Pereira EM, Leite Filho MT, de Assis MF, Martins ANA, Rocha APT (2015) Potencial toxicológico frente *Artemia Salina* em plantas condimentares comercializadas no município de Campina Grande, PB. Rev Verde Agroecol e Desenvolv Sustentável 10:52
40. Purvis A, Hector A (2000) Getting the measure of biodiversity. Nature, 405: 212–219
41. Puttaraju NG, Venkateshaiah SU, Dharmesh SM, Urs SMN, Somasundaram R (2006) Antioxidant activity of indigenous edible mushrooms. Journal of agricultural and food chemistry, 54:9764-9772
42. Putzke J, Putzke MTL (2004) Os Reinos Dos Fungos, 2a. Edunisc, Santa Cruz do Sul
43. Rajeh MAB, Kwan YP, Zakaria Z, Latha LY, Jothy SL, Sasidharan S (2012) Acute toxicity impacts of Euphorbia hirta L extract on behavior, organs body weight index and histopathology of organs of the mice and *Artemia salina*. Pharmacognosy Res 4:170
44. Ramos HP, Braun GH, Pupo T, Said S (2010) Antimicrobial Activity from Endophytic Fungi Arthrinium state of *Apiospora montagnei Sacc.* and *Papulaspora immersa*. Arch. Biol. Technol 53:629–632
45. Ribeiro B, Lopes R, Andrade PB, Seabra RM, Gonçalves RF, Baptista P, Quelhas I, Valentão P (2008) Comparative study of phytochemicals and antioxidant potential of wild edible mushroom caps and stipes. Food Chem 110:47–56
46. Schmidt-Dannert C (2014) Biosynthesis of Terpenoid Natural Products in Fungi. Biochem Eng 148:19–61
47. Shimokawa Y, Akao Y, Hirasawa Y, Awang K, Hadi AHA, Sato S, Ayoama C, Takeo J, Shiro M, Morita H (2010) Gneyulins A and B, Stilbene Trimers, and Noidesols A and B, Dihydroflavonol- C -Glucosides, from the Bark of *Gnetum gnemonoides*. J Nat Prod 73:763–767
48. Srinivasulu C, Ramgopal M, Ramanjaneyulu G, Anuradha CM, Kumar CS (2018) Syringic acid (SA)—a review of its occurrence, biosynthesis, pharmacological and industrial importance. Biomed. Pharmacother 108:547-557
49. Sunnam S, Prasad K (2013) Total Synthesis of (+)-Pinellic Acid. Synthesis (Stuttg) 45:1991–1996
50. Trott O, Olson AJ (2009) AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 31:455-461
51. Wang D, Wang J, Huang X, Ying T, Kunyi N (2007) Identification of polymethoxylated flavones from green tangerine peel (Pericarpium Citri Reticulatae Viride) by chromatographic and spectroscopic techniques. J Pharm Biomed Anal 44:63–69
52. Wu S (2009) Progresso da pesquisa sobre compostos naturais de sabor voláteis produzidos por cogumelos comestíveis. AFS, Adv Food Sci 28:54–60
53. Zapesochnaya GG, Sokol’skaya TA (1984) Spectral characteristics of C-methylflavones. Chem Nat Compd 20:284–287.
54. Zou D, Zhang A, Yan G, Tan Y, Sun H, Wang X (2014) UPLC-MS coupled with a dynamic multiple data processing method for the comprehensive detection of the chemical constituents of the herbal formula San-Miao-Wan. Anal Methods 6:2848

**Figures**
Figure 1

Representation of the percentage of inhibition of DPPH and the concentration of the methanol extract of the fungus
Figure 2

LC-MS/MS spectrum of Lentinus crinitus methanol extract in positive ion mode [M H]+
Figure 3

LC-MS/MS spectrum of Lentinus crinitus methanol extract in negative ion mode [M-H]-

Figure 4

Proposal of the structures of the compounds identified in the extract in the positive ion mode.
Figure 5

Proposal of the structures of the compounds identified in the extract in the negative ion mode.

Supplementary Files

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