Fatty ethanolamide of *Bertholletia excelsa* triglycerides (Brazil nuts): anti-inflammatory action and acute toxicity evaluation in Zebrafish (*Danio rerio*)

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Abstract

Fatty amides (N-alkylamides) are bioactive lipids that are widely distributed in microorganisms, animals, and plants. The low yield in the extraction process of spilantol, a fatty amide, which is mainly related to its diverse biological effects, compromises its application on a large scale. Thus, this study proposes an alternative method to synthesise fatty amides from *Bertholletia excelsa* (AGBe) oil, with a chemical structure similar to that of spilantol. Carrageenan-induced abdominal oedema in vivo models were used in zebrafish (*Danio rerio*). In in vivo studies, oral AGBe produced no signs of toxicity. In the histopathological study, AGBe did not cause significant changes in the main metabolising organs (liver, kidneys, and intestines). All doses of AGBe (100 mg/kg, 500 mg/kg, and 750 mg/kg) were effective in reducing oedema by 65%, 69%, and 95%, respectively, producing a dose–response effect compared to the control group, and spilantol-inhibited oedema by 48%. In the in silico study, with the use of molecular docking, it was observed that among the AGBe, the molecules 18:1, ω-7-ethanolamine, and 18:1, ω-9-ethanolamine stood out, with 21 interactions for COX-2 and 20 interactions for PLA2, respectively, surpassing the spilantol standard with 15 interactions for COX-2 and PLA2. The anti-inflammatory action hypothesis was confirmed in the in silico study, demonstrating the involvement of AGBe in the process of inhibiting the enzymes COX-2 and PLA2. Therefore, based on all the results obtained and the fact that until the dose of 1000 mg/kg was administered orally in zebrafish, it was not possible to determine the LD50; it can be said that AGBe is effective and safe for anti-inflammatory activity.

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Introduction

Ethnopharmacological studies are increasingly being conducted owing to the growing global demand for new drugs, as well as the presence of chemical components in medicinal plants, which have been associated with several pharmacological effects. Therefore, natural resources have been considered important in improving health and quality of life (Shawahna and Jaradat 2017).

Among the biologically active components reported and found in medicinal plants, we highlight the fatty amides found in abundance in Bertholletia excelsa, a tree of the Lecythidaceae family, also known as chestnut from Pará or Brazil. It is a species native to the Amazon region and is considered one of the main riches of the Amazon jungle. It is the most exported raw material in the region. The oil extracted from the seed of Bertholletia excelsa is notable for its high nutritional value and several biological activities such as healing, antioxidant, and anti-inflammatory activities (Chunhieng et al. 2008; Pena Muniz et al. 2015).

In this context, the species Acmella oleracea, a plant belonging to the Asteraceae family and found mainly in the northern region of Brazil where it is usually used in local cuisine and is popularly known as Jambú, was discovered (Dos Santos 2015). The active compound found in greater abundance in this plant is spilantol (N-alkylamide), a fatty amide with the chemical formula \(\text{C}_{14}\text{H}_{23}\text{NO}, 221.339 \text{ g/mol}\) (Molina-Torres et al. 1996; Barbosa et al. 2015). Spilantol has been used in scientific studies to demonstrate its relationship with several biological effects, such as analgesic, neuroprotective, anticonvulsant, antioxidant, and anti-inflammatory effects (Wu et al. 2008; Hernández et al. 2009; Dias et al. 2011; Silva and Oliveria 2013).

Among the various symptoms, inflammation is the most telling as it is a warning sign for the body, with prolongation of the inflammatory process causing damage to cells and tissues. However, most commercialised anti-inflammatory drugs cause adverse reactions when used in the long term. This has been one of the biggest challenges for doctors and pharmacists who develop research with products of natural origin (Aracama et al. 2000; Carvalho 2017; Shawahna and Jaradat 2017). Considering ethnopharmacology and ethnomedicine, some products of natural origin have been studied and identified as possible alternative drugs for the treatment of inflammation.
In this study, zebrafish (Danio rerio) was selected as an animal model, as it has been recognised pharmacologically for its advantages in carrying out scientific research and validating new drugs, as well as its successful application in the pharmaceutical field (Hsu et al. 2007; Chakraborty et al. 2010; Kettleborough et al. 2013; Schmidt et al. 2013; MacRae and Peterson 2015; Brugman et al. 2016; Carvalho et al. 2017; Borges et al. 2018).

The present study proposed obtaining fatty amides from Bertholletia excelsa oil and have its pharmaco-toxicological validation for anti-inflammatory activity in zebrafish (Danio rerio) in comparison with spilantol, a fatty starch extracted from the flowers of Acmella oleracea, which proved to be an effective anti-inflammatory compound.

Materials and methods

Plant materials—obtaining spilantol

Spilantol was previously obtained in a study by de Souza et al. (2020) from the plant species Acmella oleracea, and in this study, it was used as an anti-inflammatory standard for the fatty amides in Bertholletia excelsa oil.

Obtaining fatty amides (N-alkylamides) from Bertholletia excelsa oil

Bertholletia excelsa oil was purchased from the Mixed Vegetable Extractive Cooperative of Laranjal do Jari Farmers (COMAJA Co.), municipality of Laranjal do Jari, Amapá, Brazil, and were stored at – 4 °C until use. Ethanolamine (99.5%), Amano lipase from Pseudomonas fluorescens [LPF (20.000 U/g, CAS 9001-62-1)], and hexane (98%) were purchased from Synth Co. (São Paulo, Brazil).

The amidation reaction (Fig. 1) was carried out with ethanolamine (6.0 mL), BNO (2.0 mL), and 10% LPF (w/w of chestnut oil) as a catalyst, with magnetic stirring for 48 h (300 rpm, 50 ± 2 °C). After that period, the enzyme was filtered, and the filtrate was extracted with dichloromethane (3×25 mL). The organic phase was dried over anhydrous Na₂SO₄, filtered, and evaporated under reduced pressure. Finally, the expected products were purified via column chromatography with silica gel and a mixture of n-hexane and ethyl acetate (8:2) as the eluent (Barata et al. 2020).

GC–MS analysis

Fatty acids and fatty amides from Bertholletia excelsa oil were characterised with gas chromatography coupled to mass spectrometry (GC–MS), using a Shimadzu/GC 2010 apparatus coupled to a Shimadzu/AOC-5000 autoinjector and an electron beam impact detector (Shimadzu MS2010 Plus) (70 eV), equipped with a DB-5MS fused silica column (Agilent J & WAdvanced 30 m × 0.25 mm × 0.25 mm) (65 kPa). The parameters used were: 1:15 split ratio, helium as the carrier gas, injection volume of 1.0 mL, injector temperature of 250 °C, detector temperature of 270 °C, initial column temperature of 100 °C for 2 min, and rate of heating from 6 °C min⁻¹ to 280 °C for 5 min. The total analysis time was 37 min. Fatty acid amides were identified by comparing their fragmentation spectra with those in the GC–MS library (MS database, NIST 5.0) (Araújo et al. 2018).

Fig. 1 Reaction of aminolysis with Bertholletia excelsa oil and ethanolamine
Identification of fatty amides

Spectroscopic profile in the infrared region

The spectroscopic profile in the infrared region of the samples (Bertholletia excelsa oil and fatty amides) were obtained via impregnation in KBr tablets, and the sample readings were performed on an infrared spectrometer by Fourier transform (FTIR) (Shimadzu IR Prestige -21), with a wavelength of 400–4000 cm−1 and a resolution of 4 cm−1 and 64 scans.

Animal experimentation

Animals

The zebrafish (Danio rerio) of the wild strain from Aqua New Aquários e Peixe Co. (PE, Brazil) following the methodology of Souza et al. (2016) and Borges et al. (2018) was used. The experiments were carried out following the rules established for the care of animals, and the project was approved by the Ethics Committee on the Use of Animals (CEUA—UNIFAP) of the Federal University of Amapá (Protocol Number 020/2019).

Determination of the average lethal dose (LD50) and behavioural assessment

To obtain the average lethal dose (LD50), the animals were observed for 48 h after treatment. In this test, adult zebrafish (standard length, 28.1 ± 0.2 mm weighing 0.42 ± 0.04 g) were used. The animals were treated orally through gavage, as described by Carvalho et al. (2017) and Borges et al. (2018). The doses of AGBe administered were 45 mg/kg, 500 mg/kg, and 1000 mg/kg, based on previous studies by de Souza et al. (2020).

Each dose of AGBe was evaluated in groups of four animals. The tests were performed in triplicate, totalling 48 animals (n = 12 per group) distributed in the following groups: group A—PBS: PBS-phosphate buffered saline (20 μL, i. p.), substance used to solubilize carrageenan, and saline solution (2 μL, v.o); group B—Thinners: carrageenan (i.p) and solution used to dilute AGBe (Tween—DMSO and distilled water, 2 μL, v.o); group C—indomethacin: carrageenan (i.p) and indomethacin (10 mg/kg, v.o, Sigma Co. São Paulo, Brazil); group D-E (35 mg/kg, Carrageenan (i.p) and spilantol at a dose of 35 mg/kg, v.o); group E—A (100 mg/kg): carrageenan (i.p) and AGBe at a dose of 100 mg/kg (v.o); group F—A (500 mg/kg): carrageenan (i.p) and AGBe at a dose of 500 mg/kg (v.o); group G—A (750 mg/kg): carrageenan (i.p) and AGBe at a dose of 750 mg/kg (v.o).

Carrageenan-induced abdominal oedema assay

Carrageenan (iota type II, Sigma Co, Lot 65H1096) was administered intraperitoneally (i.p.) in a volume of 20 μL (200 μg) in PBS, according to the methodology described by Borges et al. (2018), 1 h before the oral administration (v.o) of AGBe. Oedema was measured at the maximum peak, which occurred 3 h after the application of carrageenan.

The trial was performed in triplicate, and the doses of AGBe were selected from the toxicity trial and based on previous studies (Collymore et al. 2013; Carvalho et al. 2017; Borges et al. 2018).

The animals were divided into the following groups with n = 16 per group: group A—PBS: PBS-phosphate buffered saline (20 μL, i. p.), substance used to solubilize carrageenan, and saline solution (2 μL, v.o); group B—Thinners: carrageenan (i.p) and solution used to dilute AGBe (Tween—DMSO and distilled water, 2 μL, v.o); group C—indomethacin: carrageenan (i.p) and indomethacin (10 mg/kg, v.o, Sigma Co. São Paulo, Brazil); group D-E (35 mg/kg, carrageenan (i.p) and spilantol at a dose of 35 mg/kg, v.o); group E—A (100 mg/kg): carrageenan (i.p) and AGBe at a dose of 100 mg/kg (v.o); group F—A (500 mg/kg): carrageenan (i.p) and AGBe at a dose of 500 mg/kg (v.o); group G—A (750 mg/kg): carrageenan (i.p) and AGBe at a dose of 750 mg/kg (v.o).

Histopathological study

At the end of the toxicological and inflammatory evaluation experiments, the animals were euthanised according to the recommendations of the American Guidelines of the Veterinary Medical Association for Animal Euthanasia (Leary et al. 2013), and the tissues were collected for histopathological analysis.

For histopathological analysis, tissue preparation and microscopic analysis of the organs analysed were based on the techniques described by Souza et al. (2016), Carvalho et al. (2017), and Borges et al. (2017). The histological changes index (IHA) was calculated from the levels of tissue changes observed in the liver, kidneys, and intestines. These alterations were classified into I, II, and III levels, with the IHA value indicating the severity of the organ, 0–10: normal organ; 11–20: mild-to-moderate organ changes; 21–50: moderate to severe organ changes; and > 100, irreversible organ damage, according to Borges et al. (2018) and de Souza et al. (2020).

Statistical analysis

To determine the median lethal dose (LD50), probit analysis was performed using GraphPad Prism software version 6.0. The results of the histopathological study were expressed as mean ± SEM and analysed using ANOVA, followed by the Tukey–Kramer test for comparisons between the treated and control groups. To evaluate anti-inflammatory activity, data were expressed as mean ± standard deviation, and analysed using ANOVA (one-way), followed by the Tukey–Kramer test.
post hoc test. Statistical significance was set at $p < 0.05$. Data were analysed using the GraphPad Prism software version 8.0.

**Molecular docking of amides in biological targets related to the inflammatory process**

For the docking study, files were deposited in the Protein Data Bank (PDB) of the Research Collaboratory for Structural Bioinformatics (Li and Chiang 2008; Sandy and Butler 2009; Orlando and Malkowski 2016) with the coordinates of the crystallographic structures of COX-1 therapeutic targets (PDB ID: 3N8X, resolution: 2.75 Å) complexed with the nimesulide inhibitor. COX-2 (PDB ID: 5IKQ, resolution: 2.41 Å) was complexed with melocenamic acid and phospholipase A2 (PDB ID: 5G3N, resolution: 1.8 Å) with 3- (5'-benzyl-2'- carbamoyl-biphenyl-3-yl) propanoic acid inhibitor.

The genetic optimisation for ligand docking (GOLD) program uses a genetic algorithm for flexible ligand docking experiments within protein binding sites. The GOLD program was used to investigate the modes of interaction between the studied compounds and therapeutic targets (Chandak et al. 2014).

For molecular coupling, hydrogen atoms were added, and the water molecules of the enzymes were removed. Inhibitors complexed with each therapeutic target were then extracted. Before performing the docking simulation, the results were validated by calculating the mean quadratic deviation (RMSD) between the experimental ligand and the conformation of the ligand that produced the best pose after docking.

The docking was calculated using the following coordinates: cyclooxygenase-1 (COX-1): x: -21.44, y: -50.78, z: 1.42, and a radius of 10 Å; cyclooxygenase-2 (COX-2): x: 22.83, y: 51.56, z: 17.81, and a radius of 9 Å; and phospholipase A2 (PLA2): x: 7.48, y: 3.41, and z: -0.16, and a radius of 9 Å. In identifying the interactions between the compounds and the therapeutic targets, it was necessary to identify the amino acids that make up the catalytic site of the enzymes: COX-1 (ARG120, TYR355, ILE523, and SER530), COX-2 (TYR385 and SER530) (Borges et al. 2017) and PLA2 (PHE5 and ILE9) (Giordanetto et al. 2016).

**Results**

**CG–MS and infrared analysis**

In the infrared region of the spectrum, the amides present (Fig. 2) a band in 3294 cm$^{-1}$ originating from stretching vibrations of the N–H bond; in 2918 and 2848 cm$^{-1}$ referring to the asymmetric and symmetrical C-H stretch, respectively; at 1643 cm$^{-1}$, an amide group C = O absorption band; in 1558 cm$^{-1}$ folding band N–H of secondary amides; and at 1468 cm$^{-1}$ was the observed bands of vibrations of asymmetric angular deformation of the C–H connections of the aliphatic groups.

In the infrared spectrum of the *Bertholletia excelsa* oil (Fig. 3), it is possible to observe bands in the region of 3007 cm$^{-1}$ referring to the stretch = −CH; in 2929 and 2854 cm$^{-1}$ referring to the asymmetric and symmetrical C–H stretch, respectively; in 1747 cm$^{-1}$ for the C = O stretch and in 1163 cm$^{-1}$ for the C-O ester stretch. At 1462 cm$^{-1}$ and 1377 cm$^{-1}$, the bands of vibrations of asymmetric angular deformation of the C-H connections of the aliphatic groups can be seen.

Oleic acid (C18:1, ω-9) is the major fatty acid contained in the *Bertholletia excelsa* oil with 32%, followed by polyunsaturated linoleic acid (C18:2, ω-6) (29%) and vaccenic acid (C18:1, ω-7) to a lesser extent at 2%. Other fatty acids identified in this oil sample were palmitic acid (C16:0; 19%) followed by stearic acid (17%). The fatty acid profile of *Bertholletia excelsa* oil is shown in Table 1, according to the reported by Barata et al. (2020).

The triglyceride characterisation by IR spectroscopy of Bertholletia excelsa oil (Fig. 2), showing a peak in 3007 cm$^{-1}$, corresponds to the stretching of the $–$C–H.
(sp2) and 2927 and 2854 cm⁻¹ to the stretching of the ligation –C–H (sp3). Amidated signals of the compound are listed in the 3295 cm⁻¹ peaks, which correspond to the stretch –NH, and the peak in the 1646 and 1567 cm⁻¹ correspond to the folding of the ligation –N–H, characteristics of the fatty amide synthesised. Another powerful signal at 1742 cm⁻¹ corresponded to the C = O stretching ester vibration; this signal is shifted to a lower frequency at 1763 cm⁻¹ for fatty amide.

The fatty amides from ethanolamine were characterised by MS spectrum with a typical base peak at m/z 116 [for N-(2-hydroxyethyl)oleamide], resulting from McLaffery rearrangement and γ-cleavage, respectively (Fig. 3). In contrast, observations of the ethyl oleate structure mass spectra (Fig. 3) showed the m/z 55 fragmentation ion as the base peak and was less abundant than the ion related to the loss of the ethoxide portion (m/z 264).

Fig. 3 Mass spectra of the ethyl oleate (A) and oleic acid amide (B) compounds
Fatty ethanolamide of *Bertholletia excelsa* triglycerides (Brazil nuts): anti-inflammatory properties

### Table 1: Composition of ethyl ester from samples derived from *Bertholletia excelsa* oil determined by GC-MS analysis

| Fatty acid<sup>a</sup> | Peak (min.) | Fatty Amide corresponding | Relative concentration (%)<sup>b</sup> |
|------------------------|-------------|--------------------------|-------------------------------------|
| Palmitic (C16: 0)      | 1           | \(\text{N-C16:0-ethanolamine}\) | 19                                  |
| Linoleic (C 18: 2 \(\omega-6\)) | 2             | \(\text{N-C18:2, \(\omega-6\)-ethanolamine}\) | 29                                  |
| Oleic (C 18: 1 \(\omega-9\)) | 3             | \(\text{N-C18:1, \(\omega-9\)-ethanolamine}\) | 32                                  |
| Vaccine (C 18: 1 \(\omega-7\)) | 4             | \(\text{N-C18:1, \(\omega-7\)-ethanolamine}\) | 2                                   |
| Stearic (C 18: 0)      | 5           | \(\text{N-C18:0-ethanolamine}\) | 17                                  |
| Not identified          | *           | –                         | 1                                   |
| \(\sum\) Saturated     | –           | –                         | 36                                  |
| \(\sum\) Unsaturated   | –           | –                         | 34                                  |
| \(\sum\) Polyunsaturated | –           | –                         | 29                                  |

<sup>a</sup>MS database (NIST 5.0)

<sup>b</sup>% of the fatty acid corresponding to the *Bertholletia excelsa* oil

### Obtaining the average lethal dose (LD<sub>50</sub>)

The animals treated with AGBe and oil of *Bertholletia excelsa* did not die during or after the experiment, including those treated with the highest dose (1000 mg/kg, v.o). Furthermore, this dose was chosen as AGBe reaches its maximum solubility for oral administration in zebrafish. The animals showed stressful behaviour in the first hours and soon recovered. Histopathological evaluation showed that both AGBe and *Bertholletia excelsa* oil did not cause tissue damage that could alter the functioning of the main organs (Fig. 4). In addition, *Bertholletia excelsa* oil was not toxic to the liver (Fig. 5).

### Carrageenan-induced abdominal oedema assay

The application of carrageenan to the animals’ peritoneum produced visible oedema, with a maximum peak observed in the third hour after application (Fig. 6A), which was inhibited by around 48% in the spilantol group. Treatment with AGBe at doses of 100, 500, and 750 mg/kg produced a dose-dependent effect, with the highest dose producing 95% inhibition (Fig. 6B). The effects of the treatments are visible macroscopically in Fig. 7.

The IHA values of the groups treated with AGBe demonstrated that the liver (5.66 ± 0.233), kidney (3.41 ± 0.258), and intestine remained functionally normal. The group treated with spilantol at a dose of 35 mg/kg produced changes in the intestine with IHA of 12.66 ± 0.05, which was significantly different from the control group (Fig. 8), with marked intestinal histopathological changes, mainly the occurrence of goblet cell hyperplasia (Fig. 10). In the...
groups treated with AGBe, the observed alterations were not decisive for the loss of tissue function (Figs. 9, 10, 11).

Molecular docking of amides in biological targets related to the inflammation process

The RMSD values obtained with nimesulide, meclofenamic acid, and 3-(5'-benzyl-2'-carbamoylbiphenyl-3-yl) propionic acid inhibitors were 0.835, 0.535, and 0.978 Å for the COX-1, COX-2, and PLA2 therapeutic targets, respectively.

Docking between therapeutic targets and spilantol compounds, 16:0-ethanolamine, 18:2, ω-6-ethanolamine, 18:1, ω-9-ethanolamine, and 18:1, ω-7-ethanolamine fatty amides, with the highest score for the COX-1 therapeutic target (Fig. 12), presented 14 interactions, with 13 hydrophobic and 1 conventional hydrogen interaction with VAL116, ARG120, VAL349, LEU352, LEU359, TYR355, TRP387, PHE518, MET522, ILE523, and LEU531 amino acids. The score observed for the best pose was 72.86.

For the 16:0-ethanolamine fatty amide, 13 interactions were identified. A total of 15 were hydrophobic, and 2 hydrogen interacted with HIS90, GLN192, VAL349, LEU352, TYR355, TRP387, ASN515, PHE518, ILE523, and ALA527. The score observed was 71.76.

The 18:1 ω-9-ethanolamine fatty amide showed 16 interactions, 14 of which were hydrophobic and 2 interacted with the amino acids HIS90, GLN192, VAL349, LEU352, TYR355, LEU384, TRP387, ASN515, PHE518, ILE523, and ALA527. The observed score value was 71.76 for the best pose.

For 18:1 ω-7-ethanolamine amide, 16 interactions were identified. A total of 15 were hydrophobic, and 1 hydrogen interacted with the amino acids HIS90, LEU352, PHE518, MET522, and ILE523. The highest score was observed at 78.40.

The 18:0-ethanolamine fatty amide showed 7 interactions, 6 of which were hydrophobic and 1 of hydrogen with HIS90, LEU352, PHE518, MET522, and ILE523- amino acids with a score value for the best position of 74.41.

Considering the amino acids from the active site, only spilantol interacted with the ARG120 residue. All molecules interacted with the amino acid ILE523. The amino acid residue TYR355 interacted with 18:2, ω-6-ethanolamine, 18:1, ω-9-ethanolamine, and 18:1 ω-7-ethanolamine molecules. However, no linker interacted with the residue SER530 amino acid.

With the COX-2 therapeutic target (Fig. 14), the spilantol and 18:2, ω-6-ethanolamine molecules showed 15 interactions, the first being 14 hydrophobic and a hydrogen interaction with VAL116, ARG120, VAL349, LEU352, LEU359, TYR355, TRP387, PHE518, MET522, and LEU531 amino acids, with 63.00 scores for the best pose. Moreover, for the 18:2 ω-6-ethanolamine molecule, 13 hydrophobic and 2 hydrogen interactions with ARG120, VAL349, LEU352, LEU384, TYR385, TRP387, PHE518, MET522, and VAL523, and LEU531 amino acids, with 63.00 scores for the best pose. Moreover, for the 18:2 ω-6-ethanolamine molecule, 13 hydrophobic and 2 hydrogen interactions with ARG120, VAL349, LEU352, LEU384, TYR385, TRP387, PHE518, MET522, and VAL523, and LEU531 amino acids, with a score of 76.71.

For the 16:0-ethanolamine and 18:1 ω-9-ethanolamine molecules, 18 interactions were observed, with 13 hydrophobic and 5 hydrogen interactions with SER119, ARG120, VAL349, LEU352, LEU384, TYR385, TRP387, PHE518, MET522, ILE523, and LEU531 amino acids. The score observed for the best position was 72.86.

For the 16:0-ethanolamine fatty amide showed 13 interactions, 11 of which were hydrophobic and 2 hydrogen with HIS90, PRO191, LEU352, TYR385, TRP387, ASN515, PHE518, ILE518, and ILE523. The score obtained for the best position was 71.53.

For 18:2, ω-6-ethanolamine fatty amide, 15 interactions were observed, 11 of which were hydrophobic and 4 hydrogen interactions with the amino acids HIS90, PRO191, GLN192, LEU352, TYR355, TRP387, ASN515, PHE518, ILE523, and ALA527. The score obtained for the best position was 77.21.

The 18:1 ω-9-ethanolamine fatty amide showed 16 interactions, 14 of which were hydrophobic and 2 interacted with the amino acids HIS90, GLN192, VAL349, LEU352, TYR355, LEU384, TRP387, ASN515, PHE518, ILE523, and ALA527. The observed score value was 71.76 for the best pose.

For 18:1 ω-7-ethanolamine amide, 16 interactions were identified. A total of 15 were hydrophobic, and 1 hydrogen interacted with the amino acids HIS90, GLN192, VAL349, LEU352, TYR385, TRP387, PHE518, MET522, and ALA527. The highest score was observed at 78.40.

The 18:0-ethanolamine fatty amide showed 7 interactions, 6 of which were hydrophobic and 1 of hydrogen with HIS90, LEU352, PHE518, MET522, and ILE523- amino acids with a score value for the best position of 74.41.

Considering the amino acids from the active site, only spilantol interacted with the ARG120 residue. All molecules interacted with the amino acid ILE523. The amino acid residue TYR355 interacted with 18:2, ω-6-ethanolamine, 18:1, ω-9-ethanolamine, and 18:1 ω-7-ethanolamine molecules. However, no linker interacted with the residue SER530 amino acid.

With the COX-2 therapeutic target (Fig. 14), the spilantol and 18:2, ω-6-ethanolamine molecules showed 15 interactions, the first being 14 hydrophobic and a hydrogen interaction with ARG120, VAL349, LEU352, LEU359, TYR355, TRP387, PHE518, MET522, and VAL523, and LEU531 amino acids, with 63.00 scores for the best pose. Moreover, for the 18:2 ω-6-ethanolamine molecule, 13 hydrophobic and 2 hydrogen interactions with ARG120, VAL349, LEU352, LEU384, TYR385, TRP387, PHE518, MET522, and VAL523, and LEU531 amino acids, with a score of 76.71.

For the 16:0-ethanolamine and 18:1 ω-9-ethanolamine molecules, 18 interactions were observed, with 13 hydrophobic and 5 hydrogen interactions with SER119, ARG120, VAL349, LEU352, LEU384, TYR385, TRP387, PHE518, MET522, ILE523, and LEU531 amino acids. The score observed for the best position was 72.86.

The 16:0-ethanolamine fatty amide showed 13 interactions, 11 of which were hydrophobic and 2 hydrogen with HIS90, PRO191, LEU352, TYR385, TRP387, ASN515, PHE518, ILE523, and ALA527. The score obtained for the best position was 71.53.

For 18:2, ω-6-ethanolamine fatty amide, 15 interactions were observed, 11 of which were hydrophobic and 4 hydrophobic interactions with the amino acids HIS90, PRO191, GLN192, LEU352, TYR355, TRP387, ASN515, PHE518, ILE523, and ALA527. The score obtained for the best position was 77.21.
Fatty ethanolamide of *Bertholletia excelsa* triglycerides (Brazil nuts): anti-inflammatory activity

**Fig. 6** A Effect of oral administration of saline and PBS (SS/PBS, 2 μl), Tween + DMSO + distilled water 2 μl (Thinners), Indomethacin (10 mg/kg), spilantol (E—35 mg/kg), AGBe (A 100 mg/kg, 500 mg/kg and 750 mg/kg) on carrageenan edema (200 μg/animal). B Inhibition percentage obtained with oral treatment with different doses of AGBe and Spilantol (E) on carrageenan edema. *p < 0.05 ANOVA followed by the Tukey test, n = 16

**Fig. 7** Macroscopic view of the effect of oral administration of saline and PBS (SS/PBS, 2 μl—A), Tween + DMSO + distilled water 2 μl (Thinners—B), Indomethacin (C—10 mg/kg), spilantol (D—35 mg/kg), AGBe (E, F, G 100 mg/kg, 500 mg/kg and 750 mg/kg) on carrageenan edema (200 μg/animal)

**Fig. 8** Effect of oral administration of saline and PBS (SS/PBS, 2 μl), Tween + DMSO + distilled water 2 μl (Thinners), Indomethacin (10 mg/kg), spilantol (35 mg/kg), AGBe (100 mg/kg, 500 mg/kg and 750 mg/kg) on the Index of Histopathological Changes for liver, intestine and kidney in Zebrafish with the application of carrageenan (200 μg/animal). Histopathological alteration index (IHA): AGBe was not toxic to the organs. Spilantol shows to be toxic to the intestine causing mild-to-moderate changes. *p < 0.05 ANOVA followed by the Tukey test, n = 16
SER353, TYR355, LEU359, PHE518, VAL523, ALA527, and LEU531 amino acid residues, obtaining 71, 16 score value.

Considering the amino acids from the active site, the interactions of the spilantol molecules, 18:2, ω-6-ethanolamine, 16:0-ethanolamine, and 18:1, ω-9-ethanolamine, with the TYR385 residue were observed. Moreover, for the SER530 amino acid residue, only the 18:1 ω-7-ethanolamine molecule interacted. This last molecule is noteworthy, because it has the most significant number of interactions and, even
Fatty ethanolamide of *Bertholletia excelsa* triglycerides (Brazil nuts): anti-inflammatory…

Though it does not interact with the TYR385 residue, it did interact with a LEU384 nearby residue. The 18:0-ethanolamine molecule showed no interaction with the amino acids of the active site.

With the PLA₂ therapeutic target (Fig. 15), the spilantol standard molecule showed 15 intermolecular interactions, 11 of which were hydrophobic and 4 of hydrogen interactions with LEU2, PHE5, HIS6, ILE9, ALA17, CYS28, GLY29, VAL30, CYS44, HIS47, ASP48, LYS62, and PHE98 amino acids with 62.45 score value.

All other studied molecules had a score value and number of interactions greater than those of the standard molecule. The 16:0-ethanolamine and 18:1, ω-7-ethanolamine molecules showed 17 interactions, being, for the first 10 hydrophobic and 7 hydrogen interactions with LEU2, PHE5, ILE9, ALA17, CYS28, GLY29, CYS44, HIS47, ASP48, LYS52, GLU55, and LYS62 amino acid residues, with an 81.18 score. For the 18:1 amide, ω-7-ethanolamine, there were 14 hydrophobic interactions and 3 hydrogen interactions, with LEU2, PHE5, HIS6, ALA17, CYS28, GLY31, CYS44, HIS47, TYR51, GLU55, LYS62, and PHE98 amino acid residues, obtaining 86.51 score value.

The 18:2, ω-6-ethanolamine molecule showed 19 interactions, 12 of which are hydrophobic and 7 hydrogen interactions, with LEU2, PHE5, HIS6, ILE9, ALA17, CYS28, VAL30, GLY31, CYS44, HIS47, TYR51, GLU55, LYS62, and PHE98 amino acid residues, obtaining 83.93 score value.

With the 18:1 ω-9-ethanolamine molecule, 20 interactions were observed, 12 of which were hydrophobic and 8...
were hydrophobic, with LEU2, PHE5, HIS6, ILE9, ALA17, CYS28, VAL30, GLY31, CYS44, HIS47, ASP48, LYS52, and GLU55 amino acid residues, with the highest score obtained for the studied molecules being 87.02.

For the 18:0-ethanolamine molecule, 15 interactions were observed, 10 hydrophobic and 5 hydrogen interactions with LEU2, PHE5, HIS6, ALA17, CYS28, GLY31, CYS44, HIS47, ASP48, and GLU55 amino acid residues, with a score of 85.25.

Considering the amino acids from the active site, all molecules interacted with the PHE5 amino acid residue. For the ILE9 residue, only spilantol, 16:0-ethanolamine, 18:2, ω-6-ethanolamine, 18:1, ω-9-ethanolamine and 18:0-ethanolamine showed intermolecular interactions. The 18:1 ω-9-ethanolamine molecule stands out for the PLA2 target with the highest score value and number of interactions, and interacts with the two amino acid residues of the active site.

With COX-2, MMA presented nine interactions with VAL344, TYR348, VAL349, TYR385, TRP387, MET522, GLY526/ALA527, and SER530 amino acid residues. PMA had five interactions with TYR355, TRP387, VAL523, and GLY526/ALA527 amino acid residues. IMA presented seven interactions with VAL349, LEU352, TRP387, MET522, GLY526, and ALA527 amino acid residues.

| Compound                      | Molecular Docking |
|-------------------------------|-------------------|
|                               | 2D                | 3D                |
| Spilantol                     | ![](image)        | ![](image)        |
| 16:0-ethanolamine             | ![](image)        | ![](image)        |
| 18:2, ω-6-ethanolamine        | ![](image)        | ![](image)        |

![Docking of compounds Spilantol, 16:0-ethanolamine, 18:2, ω-6-ethanolamine, 18:1, ω-9-ethanolamine, 18:1, ω-7-ethanolamine e 18:0-ethanolamine performing interaction with COX-1](image)
Discussion

In recent years, zebrafish have become the most used animal in laboratories, increasing their implementation in experiments and scientific research. Most of the research results are gathered in the ZFIN community—Zebrafish Information Network (Spence et al. 2008).

Zebrafish are thus replacing the use of rodents owing to several favourable characteristics. According to Souza et al. (2016), one of them is its small size, which makes the dose administered, whether in microliters (µL) or less, of the active ingredient induces a pharmacological effect. It is highly favourable for the testing of natural products, because the material used to obtain the product is not always abundant, and the extraction efficiency can be very low. In addition, 70% of genes orthologous to Homo sapiens are highly similar to humans in terms of function of the main physiological processes, with key organ systems, such as the digestive, nervous, and cardiovascular systems (Hsu et al. 2007). It largely favours the equivalence of the response to pharmacological agents between the two species (MacRae and Peterson 2015).

Innovative studies have introduced a new route of administration to assess the toxicity of possible drugs. Oral treatment (gavage) is an efficient method for assessing the toxic potential of natural or chemical substances. The histopathology of the liver, kidney, and intestinal tissues has also been evaluated (Collimore et al. 2013; Borges et al. 2018; Sampaio et al. 2018).

Animals, when in contact with any toxic substance, whether of synthetic or natural origin, may present characteristics that indicate a possible toxic effect in the short or long term. According to Ribeiro (2013), these substances can trigger changes in different systems and behaviour, and can even cause death in animals (Mathur et al. 2011).

According to Huang et al. (2014), Borges et al. (2018), and de Souza et al. (2020), zebrafish, when in contact with foreign substances, adopt patterns of stress behaviour, as observed in this study after the oral administration of AGBe; however, the studied substances did not cause damage to the tissue level of the organs analysed in the histopathological study.

In the toxicity test, it was observed that Bertholletia excelsa oil and AGBe did not show toxicity with oral
treatment (1000 mg/kg), and it was not possible to determine the LD_{50}. It is noteworthy that we decided to use this dose above the effective dose to ensure the safety of AGBe administration. A similar result was obtained by Barata et al. (2020), who reported reduced cell toxicity for fatty amides.

de Souza et al. (2020) reported that even substances of natural origin that do not cause behavioural changes or death in the zebrafish could cause internal damage in this animal, altering the normal functioning of some organs. According to the parameters presented in studies carried out by Souza
et al. (2016) and Borges et al. (2017), the rate of histopathological changes observed in this study for the kidneys and intestines of animals treated with AGBe and *Bertholletia excelsa* oil were normal, as they did not present changes that compromised the homeostatic pattern of the organs.

In this study, *Bertholletia excelsa* oil was not toxic to the liver, and the IHA was 0. This result reinforces the findings of Pawel et al. (2013) and Barata et al. (2020), who did not demonstrate toxicity to the liver and kidneys in rats, and also showed low cellular toxicity for fatty amides.

Carnovali et al. (2016) evaluated the action of fatty acid amides in zebrafish and demonstrated that they prevent the alteration of bone markers in a prednisolone-induced osteoporosis model in adult zebrafish scales, whereas their esterified forms did not. These data suggest that long-chain fatty acid amides are involved in regulating bone metabolism.

In this study, carrageenan was used as an inflammatory agent in a zebrafish model. Huang et al. (2014) validated the use of carrageenan as an inflammatory inducer in the zebrafish peritoneum and observed that i.p. injection of carrageenan produced typical symptoms of inflammation, such as swelling, and upregulated MPO, a leukocyte marker, as well as the pro-inflammatory proteins TNF-α and iNOS. They also demonstrated that local injection of carrageenan into soft tissues induces acute inflammation, and that known compounds with anti-inflammatory properties can modulate the inflammatory responses of carrageenan-injected adult zebrafish.

Thus, for the evaluation of AGBe in the inflammatory process triggered by carrageenan, the protocols of Huang et al. (2014), Carvalho et al. (2017), and Borges et al. (2018) regarding intraperitoneal carrageenan were used to induce the formation of abdominal oedema in zebrafish.

The participation of cyclooxygenase products (prostaglandins) in carrageenan oedema, especially in the second phase, has already been described in several studies (Zaa et al. 2012; Motta et al. 2013; Huang et al. 2014; Carvalho et al. 2017; Borges et al. 2018; Barata et al. 2020). In addition, non-steroidal anti-inflammatory drugs, such as indomethacin, are inhibitors of prostaglandin synthesis via COX-1 inhibition and IL-6 production (Motta et al. 2013).

The administration of carrageenan intraperitoneally produced the formation of abdominal oedema, which was more visible in animals treated with thinner/carrageenan (Fig. 6B), and treatment with different doses of AGBe (100, 500, and 750 mg/kg) orally produced an inhibitory effect on carrageenan oedema in a dose-dependent manner (Fig. 8B). These results align with those described by Barata et al. (2020) for amides obtained from triglycerides of *Bertholletia excelsa* oil, which demonstrated antioedematogenic activity on rat paw carrageenan oedema.

The fact that fatty acid amides are described as inhibitors of cyclooxygenase and lipoxygenase (Fiorucci et al. (2001) and Barata et al. (2020)), in this study we consider to support the effect of AGBe.

In this study, molecular docking was performed for AGBe and the standard anti-inflammatory drugs. This computational method is currently widely used to obtain new drugs (Du et al. 2016). It describes the mode of interaction of molecules at the enzyme or receptor site through specific fundamental interactions and predicts the binding affinity between protein–ligand complexes.
Spilantol was used as a standard in the in silico study to compare the results as it has a chemical structure similar to the studied molecules and presents a report in the literature on anti-inflammatory activity (Wu et al. 2008).

The RMSD value indicates the accuracy of the docking poses calculated by the GOLD fitting algorithm compared to the experimentally determined poses for a compound linked to a biological target. Therefore, the calculation of docking with an RMSD of less than 2 Å for a proper conformation is considered successful. Therefore, it has justified validity (Cole et al. 2005).

Prostaglandins are derived from arachidonic acid (AA) in a reaction catalysed by COX, which can exist as COX-1 and COX-2. AAs are released from the cell membrane upon neopathological stimuli. Inhibitors of this enzyme interfere with this reaction, and the disease process begins. Recently, the involvement of COX-1 in cancer and inflammation has been firmly established (Vitale et al. 2016; Hage-Melim et al. 2019).
Chunhieng et al. (2008) and Barata et al. (2020) confirmed that the polyunsaturated fatty acids present in the oil of *Bertholletia excelsa* have different fatty amide precursors, which have anti-inflammatory properties and probably act in the COX pathway, as was observed in this study. AGBe identified as 18: 1, ω-7-ethanolamine and 18: 1, ω-9-ethanolamine, present in vaccenic and oleic fatty acids, showed more significant interaction for COX-2 and PLA₂, which stood out for presenting a score and number of interactions greater than the spilantol pattern, interacting with the amino acids present in the active site or, at least, close to it in all the studied targets (Fig. 15, 16, and 17).

The AGBe in the docking between the therapeutic targets (Figs. 12 and 13) presented a higher score for the COX-1 therapeutic target, with interactions in important amino acids of this enzyme, with the 18: 1, ω-7-ethanolamine amide, presenting the highest score value of 78.40.

Spilantol was used as a standard for comparison, because it has a chemical structure similar to AGBe, and because it has anti-inflammatory activity (Wu et al. 2008) and, with the therapeutic target COX-2 (Fig. 14), spilantol and 18:2, ω-6-ethanolamine showed interactions, with amino acids with score values of 63.00 and 76.71, respectively. With the therapeutic target PLA₂ (Fig. 15), spilantol had a score value of 62.45 and the 18: 1 molecule, ω-9-ethanolamine, had the highest score value and several interactions and interacted with the two amino acid residues of the active site.

Prostaglandins are derived from arachidonic acid (AA) in a reaction catalysed by COX, which can exist as COX-1 and COX-2. After neopathological stimuli, AA is released from the cell membrane. Inhibitors of this enzyme interfere with this reaction, and the involvement of COX-1 in cancer and several inflammatory processes is already known (Vitale et al. 2016; Melim et al. 2019).

The RMSD value indicates the accuracy of the docking poses calculated by the GOLD fitting algorithm compared to the experimentally determined poses for a compound linked to a biological target. Thus, the calculation of docking with an RMSD of less than 2 Å for a conformation of fit is considered successful. Therefore, it has justified validity (Cole et al. 2005). Therefore, all AGBe studied had a score value and number of interactions greater than the standard molecule (spilantol), indicating anti-inflammatory activity related to COX-2 and PLA₂ inhibition.

Carvalho et al. (2017) demonstrated that the administration of an inflammatory agent, such as carrageenan, in the abdominal region of *Danio rerio* can cause reactions in vital organs such as the gills, liver, intestine, and kidneys. Borges et al. (2018) stated that the technique of intraperitoneal injection in zebrafish is invasive, which can easily cause damage to the organs contained in the abdominal cavity responsible for the metabolism and excretion of various substances.

The histopathological study observed that the group treated with spilantol (Fig. 8) had an IHA of 12.66 for the intestine, considering mild-to-moderate changes. de Souza et al. (2020) reported that spilantol, depending on the dose, can influence the production of histopathological damage in the intestine, liver, and kidneys in zebrafish and reported that spilantol caused irreversible damage to the intestines of animals.
In this study, the group treated with AGBe at the highest dose (750 mg/kg) and received carrageenan as an oedematogenic agent, did not present histopathological alterations in organs evaluated that could compromise the physiological functions (Figs. 9, 10, and 11), and presented 95% inhibition of the inflammatory process triggered by carrageenan in the zebrafish peritoneum (Fig. 7B). This fact may be related to the modulation of pathophysiological mechanisms triggered by carrageenan, highlighting the participation of prostaglandins in the maximum peak of oedema (Borges et al. 2018). The anti-inflammatory action hypothesis was confirmed in the in silico study, demonstrating the involvement of AGBe in the process of inhibiting the enzymes COX-2 and PLA₂.

Conclusion

The method used to obtain AGBe from Bertholletia excelsa oil was effective and, considering the results obtained in the carrageenan oedema test in zebrafish, it can be suggested that AGBe has anti-inflammatory activity, including triggering a dose–response effect. The hypothesis of anti-inflammatory action was confirmed in the in silico study, demonstrating the involvement of AGBe in inhibiting the enzymes COX-2 and PLA₂, with emphasis on the molecules 18: 1 ω-7-ethanolamine and 18: 1, ω-9-ethanolamine. In the histopathological study, AGBe did not cause significant changes to the main metabolising organs (liver, kidneys, and intestines), whereas spilantol produced mild-to-moderate changes in the intestinal tissue. Therefore, based on all the results obtained and the fact that until the dose of 1000 mg/kg, orally, in zebrafish, it was not possible to determine the LD₅₀, it can be said that AGBe is effective and safe for anti-inflammatory activity.

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Author contributions

BR obtained and characterised AGBe under the guidance of the IMF. In vivo biological assays were performed using YFQU and SFB with GCS, BLSO, and RSB. LISHM carried out the in silico study, and JCTC participated as the general coordinator of the study and reviewer of the data obtained.

References

Araújo PHF, Barata PHdS, Araújo IF, Curti IM, Amaral RR, Bereau D, Carvalho JCT, Ferreira IM (2018) Direct and solvent-free aminolysis of triglyceride from Oenocarpus bataua (Patawa) oil catalysed by AI2O3. Catal Lett 148:843–851

Barata P, Sarquis Í, Carvalho H, Barros A, Rodrigues A, Galve-Parra A, Silva E, Carvalho JC, Ferreira I (2020) Chemoenzymatic synthesis and anti-inflammatory activity of fatty acid amides prepared from Bertholletia excelsa (Brazil Nut) triglycerides. J Braz Chem Soc 1:1–9

Barbosa AF (2015) Spilantol: occurrence, extraction, chemistry and biological activities. Rev Bras 25:128–133

Borges RS, Lima ES, Keita H, Ferreira IM, Fernandes CP, Cruz RAS, Duarte JL, Velázquez-Moyo J, Ortiz BLS, Castro AN, Ferreira JV, Hage-Melm LIS, Carvalho JCT (2017) Anti-inflammatory and antialgic actions of a nanoemulsion of Rosmarinus officinalis L. essential oil and a molecular docking study of its major chemical constituents. Inflammopharmacology 26:183–195

Borges RS, Ortiz BL, Pereira AC, Keita H, Carvalho JC (2017) Rosmarinus officinalis essential oil: a review of its phytochemistry, anti-inflammatory activity, and mechanisms of action involved. J Ethnopharmacol 229:29–45

Borges RS, Keita H, Ortiz BLS et al (2018) Anti-inflammatory activity of nanoemulsions of essential oil from Rosmarinus officinalis L.; in vitro and in zebrafish studies. Inflammopharmacology 26:1057–1080

Brugman S (2016) The zebrafish as a model to study intestinal inflammation. Dev Comp Immunol 64:82–92. https://doi.org/10.1016/j.dci.2016.02.020

Carnovali M, Ottria R, Pasqualetti S, Banfi G, Ciuffreda P, Mariotti M (2016) Effects of bioactive fatty acid amide derivatives in zebrafish scale model of bone metabolism and disease. Pharmacol Res 104:1–8. https://doi.org/10.1016/j.phrs.2015.12.009

Carvalho JCT, Keita H, Santana GR, Souza GC, Santos IVF, Amado JR, Kourouma A, Prada AL, Carvalho HO, Silva ML (2017) Efeitos do veneno de Bothrops alternatus no peixe-zebra: estudo histopatológico. Inflammopharmacology 25:1–9

Chakraborty A, Devi B, Sanjebam R, Khumbong S, Thokchom EI (2010) “Estudos preliminares sobre as atividades anestésicas e antipiréticas locais de Spilanthes Acmella Murr em modelos animais experimentais. Indian J Pharmacol 42:277–279

Chandak N, Kumar P, Kaushik P, Varshney P, Sharma C, Kaushik D, Jain S, Aneja KR, Sharma PK (2014) Dual evaluation of some novel 2-amino-substituted coumarinylthiazoles as anti-inflammatory-antimicrobial agents and their docking studies with COX-1/COX-2 active sites. J Enzyme Inhib Med Chem 29:476–484

Chunhieng T, Hafidi A, Brochier J, Didier M (2008) Detailed information. Dev Comp Immunol 64:82–92. https://doi.org/10.1016/j.dci.2016.02.020

Cole SH, Carney GE, McClung CA, Willard SS, Taylor BJ, Hirsh J (2005) Two functional but noncomplementing. Drosophila tyrosine decarboxylase genes. J Biol Chem 280:14948–14955

Collymore C, Rasmussen S, Tolwani RJ (2013) Gavaging adult zebrafish. JoVE. https://doi.org/10.3791/50691

Da Hsu S, Chu CH, Tsou AP, Chen SJ, Chen HC, Hsu PWC, Wong YH, Chen YH, Chen GH, DA Huang H (2007) miRNAMap 2.0: genomic maps of microRNAs in metazoan genomes. Nucleic Acids 36:165–169 (Published online)

de Souza GC, Viana MD, Goes LDM, Sanchez-Ortiz BL, Da Silva GA, De SouzaPineheiro WB, Rodrigues dos Santos CB, Tavares Carvalho JC (2020) Reproductive toxicity of the hydroethanolic extract of the flowers of Acmea laceracae and spilanthol in zebrafish: in vivo and in silico evaluation. Hum Exp Toxicol 39(2):127–146. https://doi.org/10.1177/0960327119878257

Dias AMA (2011) Spilantol from Spilanthes Acmea flowers, leaves and stems obtained by selective supercritical carbon dioxide extraction. J Supercrit Fluids 61:1–9

Dos Santos SM (2015) Obtenção de spilantol a partir das folhas de jambu (Spilanthes Acmea (l) murr. Universidade Federal do Ceará, Grau de bacharel.
Du X, Li Y, Xia YL, Ai SM, Liang J, Sang P, Ji XL, Liu SQ (2016) Insights into protein–ligand interactions: mechanisms, models, and methods. Int J Mol Sci 17:144

Fiorucci S, Meli R, Bucc M, Cirino G (2001) Dual inhibitors of cyclooxygenase and 5-lipoxygenase. A new avenue in anti-inflammatory therapy? Biochem Pharmacol 62:1433–1438

Giordano T, Pettersen D, Starke I, Nordberg P, Dahlström M, Knerr L, Selmi N, Rosengren B, Larsson LO, Sandmark J, Castaldo M, Deker N, Karlsson U, Hurt-Camejo E (2016) Discovery of AZD2716: a novel secreted phospholipase A2 (sPLA2) inhibitor for the treatment of coronary artery disease. ACS Med Chem Lett 7:884–889

Hage-Melêm LIS, Poiani JGC, Da Silva CHTP (2019) Boylan, F. In silico study of the mechanism of action, pharmacokinetic and toxicological properties of some N-methylanthranilates and their analogs. Food Chem Toxicol 131:110556

Hernández I, Marquez L, Martínez I, Dieguez R, Delporte C, Prieto S, Molina-Torres J, Garrido G (2009) Anti-inflammatory effects of Ethanolic extract and alkalamides-derived from Heliopsis longipes roots. J Ethnopharmacol 124:649–652

Huang SY, Feng CW, Hung HC, Chakraborty C, Chen CH, Chen Y (2013) Purely olefinic Alkamides in Heliopsis longipes and Its Complexes with Substrate Analog and Inhibitor Reveal a Ligand-specific Heme Conformation Change. H.−C, Hsu. Journal of Biological Chemistry 283(5), P-Y, Whitby, F. G, L-H, Wang, Sandy M, Butler A (2009) Microbial iron acquisition: marine and terrestrial siderophores. Chem Rev 109:4580–4595

Leary S, Anthony R, Cartner S, Corey D, Grandin T, Greenacre C, Kettleborough RN, Busch-Nentwich EM et al (2013) A systematic genome-wide analysis of Zebrafish protein-coding gene function. Nature 496:494–497

Li Y. -C, Chiang C -W (2008) Structures of Prostacyclin Synthase. JCT (2016) Obtainment and study of the toxicity of perillyl alcohol nanoemulsion on zebrafish (Danio rerio). J Ethnopharmacol 224:563–578. https://doi.org/10.1016/j.jep.2016.10.037

MacRae CA, Peterson RT (2015) Zebrafish as tools for drug discovery. Nat Rev Drug Discov 14:721–731

Mathur P, Lau B, Guo S (2011) Conditioned place preference behavior in zebrafish. Nat Protoc 6:338–345

Molina-Torres J, Salgado-Garciglia R, Ramirez-Chane E, del Rio RE (1996) Purely olefinic alkalamides in Heliopsis Longipes and Acmella (Spilanthes) oppositifolia. Biochem Syst Ecol 24:27–43

Motta EV, Pinto NC, Freitas Paiva BT, Silva Aleluia GA, Silva Neto FLP, Silva HR, Keita H, Cruz RAS, Sánchez-Ortiz BL, Pineda-Peña EA et al (2018) Leaves of Spondias mombin L. a traditional anxiolytic and antidepressant: Pharmacological evaluation on zebrafish (Danio rerio). J Ethnopharmacol 224:563–578. https://doi.org/10.1016/j.jep.2018.05.037

Peña EA et al (2018) Leaves of Spondias mombin L. a traditional anxiolytic and antidepressant: Pharmacological evaluation on zebrafish (Danio rerio). J Ethnopharmacol 224:563–578. https://doi.org/10.1016/j.jep.2018.05.037

Porres Aracama JM, Alberdi Odrozollo F, García Urria F, Marco Garde P, Rekondo Andueza YM (2000) Ablación de la unión auriculoventricular en la fibrilación auricular refractaria a tratamiento farmacológico. Med Intensiva 24:8–13

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