Synthesis, Biological Activity, and In silico Study of Bioesters Derived from Bixin by the CALB Enzyme

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Abstract: Biocatalysis is a branch of biotechnology that aims at the chemical transformation of a compound by using enzymes of known specificity. There are already several studies that use combinations of organic origin and enzymes as catalysts. The enzymatic sources are diverse and can be found in microorganisms, animals, vegetables, or commercial (enzymes isolated). The enzyme Candida antarctica lipase B (CALB), of microbial origin, commercially available and with high catalytic activity, can perform esterification reactions, obtaining expressive results. These biocatalytic reactions can contribute to the development of energy products, such as biofuels and pharmaceuticals, such as cosmetics and pharmaceuticals. In this context, the present project aims to esterify with the enzyme Candida antarctica lipase B (CALB), a natural product known as bixin, a dye extracted from the pericarp of annatto seeds (Bixa orellana L.) to verify its biological properties and in silico evidence of its cholesteric activity.

Keywords: biocatalysis; bioesterification; bixin.

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1. Introduction

Bixin is the pigment of annatto present at the top of the achiote seed (Bixa orellana), a rapidly growing shrub that reaches 4-6 meters high, robust in showy appearance flowers whose color depends on the variety. An arillus is observed on its seed surface that has several substances besides the characteristic red pigment. This arillus represents about 5% to 10% of the weight of the seed, of which the carotenoid bixin represents only 30%. Fig. 1, of reddish-yellow color and liposoluble, being a monomethyl ester of a cis-norbixin carboxylic acid having nine conjugated oleiphic bonds, with one in the cis [1–3].
This molecular structure has an isoprenoid chain (alternating double bonds), naturally with cis geometric isomers in C-16, having a carboxylic acid group and an ester group at each chain; bixin is formed by oxidative degradation of C40 carotenoids. This substance is known for its stability to light, oxidation, pH changes, temperature up to 100°C [4]. This makes it possible to perform bioreactions by enzymatic means [5].

The term biocatalysis covers the process in which a biological catalyst is used to convert a substrate into a limited number of enzymatic steps [6, 7]. These enzymes act as specific and chiral catalysts due to their high versatility in performing various types of organic reactions [8]. Biocatalytic reactions are usually safe and may occur in mild conditions of temperature, pH close to neutral, thus minimizing isomerization, racemization, and epimerization of stereogenic centers, which are frequent when using conventional catalysts [9, 10].

Lipases can be animal (pancreatic, hepatic, and gastric), microbial (bacteria and fungi), and plant origin, with variation in their catalytic properties. They are considered one of the enzymes most used in biocatalytic processes. Lipases of microbial origin are still the most used. However, the study of plant sources (stem, leaf, and latex) has grown in recent years [11, 12].

In addition to hydrolysis, lipases can also catalyze other types of chemical reactions such as esterification, transesterification (interesterification, alcoholysis, and acidolysis), aminolysis (amide synthesis), and lactation [13–15].

Currently, the immobilized enzyme Candida antarctica lipase B (Novozym 453 or CALB), of microbial origin, is considered one of the most used in bioprocesses because it has high catalytic activity [16]. Recent articles [17] report this enzyme’s use in the catalysis of the esterification reaction of various substrates, obtaining very satisfactory results. Therefore, there are also reports of enzymatic hydrolysis of fatty acid esters of red pepper carotenoids (Capsicum annuum L.) and golden flowers (Tagetes erecta L.) by this lipase [18, 19]. This enzyme showed a higher esterification index with these acids uncommon in nature than linear chain acids, such as myristic acid (C14:0) [20, 21].

Because it is easy to extract and obtain, recent studies have reported several biological and pharmacological activities of Z-bixin and its derivatives [22–24]. Including in vitro evaluation of the inhibitory activity of the enzyme acetylcholinesterase (AChE) [25, 26].

Alzheimer’s disease (AD) is a neurological pathology of progressive degenerative nature that results in impaired memory and behavior [27]. The treatment strategies are based on the cholinergic hypothesis, which postulated that memory deficiencies in patients suffering from this disease result from a deficit of cholinergic function in the brain. Cholinergic neurotransmission is significantly affected in patients with Alzheimer’s disease [28]. One of the most promising approaches to treating this disease is to increase the level of acetylcholine in the brain using acetylcholinesterase inhibitors (AChE) [29–31]. Therefore, AD’s clinical treatment involves reversible cholinesterase inhibitors in clinical trials to treat Alzheimer’s disease [30, 32].
Anticholinesterase may interact with the central cholinergic system to improve patients’ memory and cognitive deficits by decreasing acetylcholine’s degradation at the synaptic site of the brain. However, the therapeutic window is small, and the acetylcholinesterase inhibitor effect (AChE) test on erythrocytes has been proposed to guide the efficacy and safety of putative therapies [30].

In medicinal chemistry, these simulations involve representing and manipulating active chemical structures with the primary objective in the rational planning of drugs [33]. Molecular modeling systems have been developing over the years, thanks to technological evolution and computer graphics, considering that the results obtained are converted into images that significantly improve object analysis [34, 35].

The calculation methods are based on theoretical chemistry being divided into Molecular Mechanics (MM), Quantum Mechanics (QM), and molecular anchorage that helps us understand the relationship of structure with biological activity [36]. For in silico tests, it is widespread to use more than one method to describe a process for optimizing a linker or receiver [35].

Molecular Mechanics (MM), also known mathematically by the force field method, is commonly used in obtaining macromolecular and enzymatic models, given the shorter computation time required in the process [37]. We can highlight that it is a simple method that is based on the relativistic positions of atoms with the nucleus, determined by the forces of attraction and repulsion that act on the structure, being the total potential energy (E_{total}) the sum of all energies that operate in the system [38, 39]. Calculations performed by Quantum Mechanics (QM) use resolutions in the wave function of the Schroëdinger equation [40–42]. For small molecular systems, the process takes into account the Bohr-Oppenheimer approximation [43], calculating the wave functions of the nucleus and electrons separately (ab-initio), which requires a high computational cost and time [44]. Using experimental (semiempirical) parameters in the equation’s resolution facilitates some integrals. It makes it possible to find results closer to the actual value [45].

Molecular docking is widely used in molecular modeling studies applied to biomolecule analysis and drug development [46]. The method simulates a flexible ligand optimized with a rigid receptor by the genetic process [47] with an active site defined by a parameterized grid with specific coordinates and space adjusted to obtain a better conformational posture of the receptor-ligand complex [48].

Therefore, this work aims to present biocatalysis (via immobilized enzyme Candida antarctica lipase B), biological activity, and a brief in silico study of bixin bioderivatives against the ache enzyme molecular anchorage using galantamine® [49–51] as a positive pattern.

2. Materials and Methods

2.1. Botanical material.

The plant material was collected and washed with distilled water, crushed in a blender (Philips®), and stored 1000 mL beaded. Soon after, left dry in greenhouse incubator EIP-010 (Caltech®) with circulating air to avoid saturation with water vapor released from the drying material and exposed to a constant temperature of 40°C for 48 hours for drying.
2.2. *Botanical identification.*

The samples of annatto seeds were acquired, in Fortaleza, in the garden of medicinal plants, in Ceará. Professor Iracema Loiola authenticated the type. The specimen, whose exsiccate, was deposited in the Prisco Bezerra Herbarium (EAC) in the Department of Biology, Federal University of Ceará (UFC) with the #EAC0059302 voucher.

2.3. *Extraction of bixin.*

From the adaptation of the literature [52], and extraction was made by organic solvent, which is repeated five times with the same material. The solvent used was gradient between chloroform (CHCl₃) and n-hexane (C₆H₁₄) in the proportion of 1:1 in a volume of 700mL, where the material with mass 576.40g, where it was immersed around 48 hours in the solution, was subsequently performed a simple filtration using a steel sieve to remove the grains, and immediately after a vacuum filtration to remove the suspended solids from which a reddish-colored solid was obtained. Soon after, the solution was extraction, by a rota-evaporator under reduced pressure, getting a reddish-colored extract.

2.4. *Products obtained by bioreaction.*

The reaction mixture was submitted to the following processes: fractionation in filter chromatographic column, separation of constituents by column chromatography, and finally, purification by recrystallization.

2.5. *Commercial enzyme testing.*

The initial tests were performed with the immobilized enzyme *Candida antarctica* lipase B (Novozym 453 or CALB), microbial origin, commercially available, and high catalytic activity.

2.6. *Enzymatic catalysis.*

The methodology used for enzymatic catalysis was an adaptation [53, 54]; a reaction was made in a water bath at 200 rpm, at 40°C for 8 hours. Four alcohols (methanol, ethanol, propanol, and butanol) were used, as shown in Scheme 1. In each sample, 50 mg of bixin, 10 mg of CALB enzyme (Novozym 435), and 20 μl of the donation alcohol were added.

![Scheme 1. Reaction of bixin (1) with lipase.](image-url)
2.6.1. Yield from the acidity index ($A_i$).

The yield from the acidity index ($A_i$) was used to determine whether biocatalysis occurred. The methodology used was an adaptation of the literature [55, 56], where a 70% commercial alcohol neutralization was performed with sodium hydroxide (NaOH) 0.1 mol. L$^{-1}$. Soon after, a simple titration of all bioproducts was made. The acidity index calculation is described in Equation 1, while the conversion values in esters were determined from Equation 2.

\[
A_i = \text{molarity } (\text{NaOH}) \times \text{MM sample} \times F \times \frac{V_{\text{titration}}}{m_{\text{reactional}}} \quad \text{Equation 1.}
\]

\[
\text{Conversion} \% = \frac{A_i - A_{ib}}{A_{ib}} \times 100 \quad \text{Equation 2.}
\]

2.6.2. Absorption spectroscopy in the infrared region.

The absorption spectra in the infrared (IR) region were obtained from the samples’ arrangement solubilized in CH$_3$OH in potassium bromide (KBr) tablets. The analyses were performed on an FT-IR 100 PerkinElmer spectrophotometer in the region from 4000 to 650 cm$^{-1}$ at the Northeast Mass Spectrometry Laboratory, belonging to the Department of Organic and Inorganic Chemistry of the Federal University of Ceará.

2.7. In vitro tests.

2.7.1. Antioxidant activity.

The extract and products from enzymatic catalysis were diluted in methanol at seven different concentrations.

The methodology used was an adaptation [57, 58]. Each test tube contained 2 mL of sample, then 2.0 mL of (2,2-diphenyl-1-picryl-hydrazyl) DPPH solution (60μM) in methanol was added to the samples in the absence of light, where the reaction process waited 30 minutes. The samples were analyzed in the T80 UV/VIS model spectrometer in triplicate [59].

2.8. Ecotoxicity

To determine the ecotoxicity of bixin and its bioderivatives in the biological environment, the lethal dose of the same was selected, the literature methodology was adopted [60], and adaptations [61] with the use of nautilus of Artemia salina. The method was divided into three steps:

2.8.1. Stage: bioassay with Artemia salina.

Initially, it was necessary to prepare a saline solution (NaCl), in the proportion 30g/L, the pH was reappropriated with a solution of sodium hydroxide (NaOH) 5%, leaving its pH at 8; after the preparation of the solution, we used a beaker of 2.00 L, being the container externally coated with white A4 paper and aluminum foil, to allow the best visualization of the larvae. Therefore, the saline solution previously prepared in a beaker, about 1.50 L, was added, then about 50 mg of cysts of the species Artemia salina were added to the solution. The lighting of Artemia salina in the solution was by a 5W led lamp positioned under the container to be well lit. The incubation period was around 48 hours.
2.8.2. Step: preparation of samples with *Artemia salina*.

After the nauplius hatching, a Pasteur pipette was used to capture and transfer ten larvae to test tubes, which contained 4.0 mL of the extract in its interior diluted in the same saline solution prepared for hatching. The tests were performed in triplicate for each extract/compound concentration of this analysis. The concentrations used for each sample were (1.00; 0.50; 0.250; 0.125; 0.062; 0.031 mg. mL\(^{-1}\)), and all samples were dissolved in the same saline solution of cultivation in decreasing concentration. Using the method to calculate the lethal dose (LD), referring the extract or compound of low toxicity when the lethal dose is less than 50 % (LD\(_{50}\)) greater than 500 mg.mL\(^{-1}\); Moderate toxicity to LD\(_{50}\) between 100 and 500 μg.mL\(^{-1}\) and quite toxic when this value of LD\(_{50}\) is less than 100 μg.mL\(^{-1}\), according to a study in the literature [62].

2.8.3. Step: count of *Artemia salina*.

The counting of this biological assay, made through the first 24 and 48 hours after pipetting the larvae in the test tubes with solutions/extract in the respective concentrations, considering the living nauplius all those who presented movements inside the box by attraction by light, and dead to those who were at the bottom of the pipes without any action. An augment glass was used for better visualization and naupliar counting.

2.9. *In silico* study.

2.9.1. Molecular simulation.

The simulation was performed using the code AutoDock Vina [63], where it was also used for optimization and calculation of visualization AutoDock Tools [64], Avogadro [65], and the viewer Discovery Studio [66].

2.9.1.2. CALB.

The obtaining of lipase B from *Candida antarctica* was made in the Protein Data Bank (https://www.rcsb.org/structure/1TCA) repository, where it was deposited with the code 1TCA, which was generated from X-ray diffraction, with a resolution of 1.55 Å, R-Value Free: 0.157, Space Group: P 21 21 21, with a unit cell with a = 62.1 Å, b = 46.7 Å, c = 91.1 Å, α = 90°, β = 90°, γ = 90°, being classified as hydrolase enzyme, organism: *Moesziomyces antarcticus*, consisting of 1 chain (A) [67].

2.9.1.3. AChE.

The enzyme of acetylcholinesterase was also made in the Protein Data Bank (https://www.rcsb.org/structure/4EY6) repository, where it was deposited with the code 4EY6, which was generated from X-ray diffraction, with a resolution of 2.4 Å, R-Value Free: 0.206, Space Group: P 61 with the unit cell with a = 104.975 Å, b = 104.975 Å, c = 323.4 Å, α = 90°, β = 90°, γ = 120°, being classified as hydrolase enzyme, organism: *Homo sapiens*, expressed in *Homo sapiens*, consisting of 2 chains (A, B) [68].
2.9.2. Obtaining the bixin ligand and its derivatives.

The Z,E-bixin ligand was obtained from the ZINC15 repository. It was deposited with the code ZINC4097700. The other ligands: bixyl methanoate, bixyl ethanoate, bixyl propanoate, and bixyl butanoate, were constructed in Chem3D software [69, 70].

2.9.3. Optimization of structures.

The functions used in determining molecular structures’ energy are predominantly based on two theoretical approximations: the classical approximation, which includes the methods of molecular mechanics and molecular dynamics, and the quantum approximation, which provides for the *ab initio* and semiempirical methods [71]. Structural optimization and electronic characterization of the molecule, it was performed using the molecular mechanics method. This method is used to optimize the geometry of structures because it is helpful to simulate large systems up to thousands of atoms and calculate various thermodynamic and kinetic properties. The results will be the values of the total energies. These results will be analyzed to propose which structure is the most stable [72]. All optimization calculations were performed in the Software Avogadro®, a software that allows performing theoretical calculations (optimization of geometry, energy, and properties) at the level of theory MM, EHT, AM1 / PM3, and MNDO / ZINDO [65, 73, 74].

2.9.4. Molecular docking.

Molecular fixation of the bixin ligand and its derivatives to CALB lipase receptors, to verify the catalytic triad of the biocatalytic process, and AChE, to show evidence of inhibition, were performed using the AutoDock Tools graphical interface (4.2.6) that serves AutoDock Vina (1.1.2) [64, 75], parameterized with the grid value box according to the natives found in the protein in its co-crystallized form by x-ray diffraction. The two-dimensional images of the interactions and three-dimensional visualization of the complex formed were generated using Discovery Studio 2020 [76].

3. Results and Discussion

3.1. Extraction of material.

About 0.1 g of material was obtained after rota-evaporation. The total extraction yield was 5.36%.

3.2. Yielded of bioesters.

The equation shown in the methodology was used for the percentage of yield of products resulting from biocatalysis, as shown in Table 1. Thus, the following yields were obtained: bixyl methanoate 23.8%, bixyl ethanoate 13.3%, bixyl propanoate 7.3%, and bixyl butanoate 3.2%. As it was observed, the product that obtained the best yield was bioesterification with methanol, which demonstrates that the larger the chain is its mass bioconversion [77].
### Table 1. Yield of bioderivatives.

| Compound       | Mass bioconversion (%) |
|----------------|------------------------|
| Bixyl methanoate | 23.8%                  |
| Bixyl etanoate  | 13.3%                  |
| Bixyl propanoate| 7.3%                   |
| Bixyl butanoate | 3.2%                   |

#### 3.3. Characterization by FTIR spectroscopy.

An analysis was performed by infrared spectrometry with Fourier transform to characterize bixin and its derivatives (Fig. 2). These vibrational modes presented are compatible with carotenoids.

The infrared spectrum of the bioproducts showed bands ranging from 3402 cm\(^{-1}\) to 3429 cm\(^{-1}\) related to the vibration of axial O-H deformation of the acid carboxyl group.

The methyl group’s asymmetric axial deformation was reported in the region with peaks around 2360-2368 cm\(^{-1}\). Bands around 2941 to 2920 cm\(^{-1}\) were related to axial deformation of the olefinic C-H bond. The band around 1710-1730 cm\(^{-1}\) is due to the axial deformation vibration of group C=O. The band around 1616-1620 cm\(^{-1}\) was related to axial deformation of the C=C bond.

The bands between 1377-1388 cm\(^{-1}\) represent methyl group deformation, and between 1438 cm\(^{-1}\) and 1456 cm\(^{-1}\), the angular deformation of the O-H bond of the carboxylic acid group. The bands between 1264-1288 cm\(^{-1}\) refer to the axial deformation of the C-O bond of carboxylic acids. Axial deformation vibration of the C-O bond is also represented in this spectrum in 1159-1162 cm\(^{-1}\). The band at 1039-1031 cm\(^{-1}\) is due to the axial deformation vibration of the C-O bond in the esters’ O-C-C arrangement derived from primary water sources.

Finally, the vibrational modes of angular deformation outside the plane of the olefinic C-H bond appeared at 713 cm\(^{-1}\) due to the absorption of radiation from the double arrangement in the cis geometry; the other vibrations around 580, 555, and 553 cm\(^{-1}\) refer to the different vibrational modes of the C-H bond of an olefinic nature [78].

#### 3.4. Antioxidant activity.

In the antioxidant activity by DPPH free radical sequestration, a relationship between activity-concentration was observed in each sample, as shown in Table 2. Where it was possible to visualize the percentages of antioxidant potential with a variation of 71.7-3.6% for bixyl butanoate, with IC\(_{50}\) of 0.021mg/mL and for the ester that presented the lowest activity, bixyl ethanoate, with variation between 33.2-0.6%, with IC\(_{50}\) of 0.05mg/mL. For the other esters, a structural relationship was not observed based on their activity, as shown by bixin itself, bixyl methanoate, and bixyl propanoate, which had the values of IC\(_{50}\) very close to 0.0227mg/mL, 0.0272 mg/mL, and 0.0276 mg/mL. Previous studies have shown that the larger the alkyl chain in phenolic compounds, the greater its molecular stability, due to its inductive donor effect of the alkyl groups, in the case of bixyl butanoate, which may have influenced the results presented [79].
3.5. Ecotoxicity activity.

After 24 and 48 hours, the percentage of mortality of *Artemia salina* larvae was calculated in concentrations of bioproduct samples and lethal dose (LD$_{50}$) using probit log-dose regression [80] in triplicate. For the toxicity scale, extremely toxic samples are considered when their lethal dose of 50% (LD$_{50}$) has values below 1 ppm; highly toxic when the value of LD$_{50}$ is between 1 to 50 ppm; moderately toxic when the value of LD$_{50}$ passes between 50 and 500; slightly toxic when the value of LD$_{50}$ is between 500 and 5000 ppm; and practically non-toxic when the LD$_{50}$ is above 5000 ppm [62].

### Table 2. Antioxidant activity results.

| Conc (mg/mL) | bixin | Bixyl methanoate | Bixyl ethanoate | Bixyl propanoate | Bixyl butanoate |
|-------------|-------|------------------|-----------------|------------------|----------------|
| 0.03125     | 54.649| 0.411            | 57.9            | 0.093            | 33.2           |
| 0.015625    | 51.782| 0.437            | 28.3            | 0.158            | 20.8           |
| 0.007813    | 32.635| 0.440            | 8.2             | 0.202            | 19.8           |
| 0.003906    | 21.389| 0.460            | 2.6             | 0.214            | 18.1           |
| 0.001953    | 16.097| 0.590            | 1.7             | 0.216            | 0.6            |
| IC$_{50}$   | 0.022709| 0.027258 | 0.050238 | 0.027669 | 0.02134 |

3.5. Ecotoxicity activity.
Initially, the lethal dose (LD$_{50}$) data for bixin after the first 24 hours were 250.377 ppm, and after 48 hours, it presented 500.208 ppm, considering them as moderately toxic. For bixyl propanoate, an LD$_{50}$ of 470.40 ppm was obtained in the first 24 h and then 952.30 ppm for 48 h later, which was slightly toxic. Both bixyl butanoate and bixyl ethanoate got a lethal dose in the first 24 hours of 2,266.10 ppm/1,865.20, up to 48 h with 4,855.60 ppm/3,680.30 ppm, and was also slightly toxic. Only bixyl methanoate was somewhat toxic in the 24 h with LD$_{50}$ of 3,177.40 ppm and after 48 h with an LD$_{50}$ of 6,828.50 ppm, considered non-toxic. Despite the experimentally presented moderately toxicity results, previous reports on liver and kidney samples from rats demonstrated bixin as non-toxic [80]. Therefore, no clinical, behavioral, necroscopic, and histological alterations were observed. Under the study conditions, bixin produced no toxic effects on exposed animals [81]. These results are presented in Table 3 and Fig. 3.

### Table 3. Results of ecotoxicity of bioproducts in 24 and 48h.

| Esters                | LD$_{50}$ (ppm) 24h | LD$_{50}$ (ppm) 48h | Ecotoxicity          |
|-----------------------|----------------------|---------------------|----------------------|
| Bixyl methanoate      | 3,177.40             | 6,828.50            | slightly toxic/non-toxic |
| Bixyl butanoate       | 2,266.10             | 4,855.60            | slightly toxic        |
| Bixyl ethanoate       | 1,865.20             | 3,680.30            | slightly toxic        |
| Bixyl propanoate      | 470.40               | 952.30              | slightly toxic        |
| Bixin                 | 250.377              | 500.208             | moderately toxic      |

**Figure 3.** Ecotoxicity of bixin and its derivatives.

### 3.6. In vitro study.

3.6.1. Result of bioesterification analysis by molecular docking of bixin with lipase (CALB).

Based on the literature [82] and with some adaptations [83], this molecular coupling simulation study was used to elucidate the transesterification interactions between bixin and lipase in the formation of four esters.

The coupling molecule by Autodock vina made it possible to list the ligands' affinity and RMSD energies, as shown in Table 4.

### Table 4. Result of molecular coupling with its energy and RMSD.

| Carotenoid | Energy affinity (Kcal/mol) | RMSD |
|------------|----------------------------|------|
| bixin      | -5.4                       | 2.2  |
Generally, the catalytic triad of a lipase consists of Ser-His-Asp. The simulation suggested that Serina 105, Histidine 224, and Aspartic Acid 187 are typically protected by a hydrophobic helical cap called “lid” [84, 85].

This enzyme presented a much-needed residue in the hydrophilic interaction, effectively activating esterification reactions for ester production, Thr 158 [86]. In addition to this residue, a hydrophobic interaction of Leucine 278 was observed [87].

The reactions of bixin ester production occurred with the formation of bixyl alkylates, as shown in Fig. 4.

![Figure 4](https://biointerfaceresearch.com/)

**Figure 4.** The mechanism by docking bixin (red) with lipase amino acid residues (blue) and catalytic triad (green) visualized by Discovery Studio 2020 [76].

The simulation was chosen to perform molecular anchoring blindly [88], where the grid was established throughout the enzymatic region. Standard practice is when one does not have a native file of the co-crystallized file obtained by X-ray diffraction. It is possible to check-in Fig. 5 the bixin with their respective amino acid residues of lipase. There is a hydrophilic approach to the Thr 158 residue making a hydrogen bond 2.8 Å away. Also, four approaches of hydrophobic nature of the alkyl type were observed: one in the residue Ileu189 in two regions of bixin, with 4.5 and the other with 3.9 Å distance, in Ala279 with 5.1 Å, Ala282 with 2.8 Å, and in Leu278, in three regions with the spread between 2-3 Å.

![Figure 5](https://biointerfaceresearch.com/)

**Figure 5.** 2D interactions of bixin with CALB with proper distances in Å at Discovery Studio 2020 [76].

3.6.2. Result of bixin and its derivates simulation by molecular docking against the AChE enzyme.

Based on the simulation of bixin and the other bioesters, with AutoDock Vina [63], against the enzyme AChE and using galantamine as a comparison, it was possible to verify that
bixin presented better receptor-binder interaction on its bioderivatives (Table 5). With a minimal difference in energy affinity level (kcal/mol) and its mean square deviation (RMSD) ratio, excluding only bixyl butanoate that had lower stability with affinity energy of -6.0 kcal/mol, compared to the others, as shown in Table 6.

**Table 5.** Comparison of interactions of ache enzyme residues between galantamine and bixin.

| Residues | Distance (Å) | galantamine | bixin |
|----------|--------------|-------------|-------|
| Trp86    | 4.32/5.24    | 3.50/5.48   |
| Gly121   | 4.13         | 3.61        |
| Tyr124   | 3.46         | 3.76        |
| Tyr337   | 5.33         | 5.49/5.20   |
| Phe338   | 4.82         | 5.11        |
| His447   | 3.06/5.15    | 5.49        |

**Table 6.** Data from the simulation of bixin and bioderived esters.

| kcal/mol | Esters          | RMSD |
|----------|-----------------|------|
| -9.1     | Bixin           | 1.6  |
| -8.7     | Bixyl ethanoate | 2.3  |
| -8.6     | Bixyl methanoate| 2.4  |
| -8.3     | Bixyl propanoate| 1.7  |
| -6.0     | Bixyl butanoate | 2.9  |

This behavior can be caused by a four-carbon alkyl chain, which tends to increase the lipophilicity of the bioester and decrease its interaction with the enzyme.

In Figure 6. It is shown that bixin (red) is linked in the same region of galantamine (orange) ligand, which is currently one of the drugs widely used to inhibit acetylcholine [89]. It can also verify two hydrophilic interactions in Tyr124 and Gly121 residues, with hydrogen bonding by carbonyl. In these four hydrophobic interactions, two Pi-alkyl types are perceived in Tyr337 residue, a significant segment, the His447 and Phe338 residues, responsible for inhibiting the enzyme [89, 90], and one of the Pi-sigma types, in the residue Trp86.

**Figure 6.** Bixin and derivatives (red) with AChE and its amino acid residues (green) and the native used binder Galantamine® (orange), visualized by Discovery Studio 2020 [76].

### 4. Conclusions

After carotenoid (bixin) has been extracted from the annatto seed employing a binary mixture of organic solvents, it was possible to perform an enzymatic catalysis reaction to obtain the bioderivatives: bixyl butanoate (3.2%), bixyl propanoate (7.3), bixyl ethanoate (13.3) and
bixyl methanoate (23.8), proving the versatility of CALB lipase despite low mass conversions. After obtaining these bioproducts, it was verified that its antioxidant and ecotoxicity levels, where bixyl butanoate presented a higher antioxidant capacity, with 71.7% in its highest concentration lower level of ecotoxicity, with slightly toxic classification. Through molecular anchorage simulation with the acetylcholinesterase enzyme, bixin showed the best receptor-binding interaction compared to its bioderivatives and the positive commercially used pattern, galantamine, with affinity energy -9.1 kcal/mol. This study contributes to the search for new compounds in the face of Alzheimer’s disease.

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Conflicts of Interest

The authors declare no conflict of interest.

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