Chemical Composition, Antibacterial Potential and Enzymatic Inhibition of the *Hedyosmum brasiliense* Mart-Chloranthaceae
ABSTRACT

The *Hedyosmum brasiliense* Mart. is a native species very occurring in the Paraná coastal region. In this study, we have available antibacterial and enzymatic inhibition assays of the volatile oil of the species. The material was collected in the Saint-Hilaire /Lange-Matinhos – Paraná - Brazil National Park. The essential oil was obtained by hydrodistillation in the Clevenger type graduated apparatus. A total of 14 compounds were identified, among them *Biciclogermacrene* was the major component. The antibacterial assay showed that essential oil was active against *Staphylococcus aureus*. However, it was inactive against *gram-negative bacterium Escherichia coli*. In the enzymatic inhibition assays was observed that the essential oil inhibited the enzyme acetylcholinesterase, whereas it was not active to the *α*-glucosidase. The results of the biological activity studies of *Hedyosmum brasiliense* Mat. are promising to recognize their potentialities.

**Keywords:** Acetylcholinesterase and *α*-Glucosidase, *in Vitro* Assays, *Hedyosmum Brasiliense*, Volatile Oils.
INTRODUCTION

Studies aimed at finding new potential species for the cure and treatment of diseases have reached a wide range and diversification in recent years. In this sense, the new potential discoveries have aroused interest in the pharmaceutical industry to manufacture new drugs from natural sources.

In this respect, it should be noted that the Atlantic Forest is one of the forest formations with the greatest biological potential in Brazil. Among the wide diversity in this biome, the *Hedyosmum brasiliense* Mart. species, stands out because it is extracted and managed for medicinal use. *Hedyosmum brasiliense* Mart. belongs to the family *Chloranthaceae*, which is constituted by 4 genera with 80 species. The leaves of the species are more or less fleshy, aromatic, with loose petiole sheath and free part of the petiole of 0.7-1.6 cm; lamina glabra on both sides, 8-9 cm long by 2.5-5.0 cm wide (LORENZI, p.79, 2016). The tree is very common in the Paraná coastal region. The plant popularly known as “Cidreira do Mato” belongs to the *Chloranthaceae* family and it has different characteristics, such as: dioecious plant species (female and male sex in different individuals); heliophyte (dependent on sun exposure); height of approximately 3-6 meters; aromatic leaves and secretory glands of essential oils present in the leaves. However, flowering of the *Hedyosmum brasiliense* Mart. occurs between the months of August and November and the fruiting of the species appears between the months of December to March. Inflorescences can be characterizing as axillary and terminal, the male ones composed of 3-8 ears and the female paniculated and surrounded by fleshy floral bracts joined at the base. The fruit is constituted by globose drupe, slightly trigonal, milky, with persistent chalice surrounded by fleshy bracts (LORENZI, 2016, p.79). Figure 1 shows the species *Hedyosmum brasiliense* Mart. in the Atlantic Forest of the Paraná-Brazil coastal region.

**Figure 1.** Native species *Hedyosmum brasiliense* Mart. in the Atlantic Forest (National Park Saint-Hilaire / Lange-Paraná-BR)
It is important to point out that the *Chloranthaceae* family appears in specific places, and it is little found. Phylogeny studies as report by Souza & Lorenzi (2005) suggested that this family is relatively isolated and can be considered as the only representative of a separate order or not to be included in any order (SOUZA & LORENZI, p. 65, 2005). In addition, *Chloranthaceae* family possesses characteristics of arboreal, median plants and cells of essential oils.

The essential oils of the species are also used in phytochemical research. Antimicrobial responses against *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* was carried out which results have shown (Vido, 2009). Besides, studies have also shown that species presents analgesic, antifungal and antioxidant activities (Furtado, 2018). Ferreira & Alves (2008) evaluated the diuretic activity of *Hedyosmum brasiliense* Mart. in which it was possible to verify their efficacy and increase of diuresis without alteration of the arterial pressure. This finding validated the indication for renal troubles treatments.

Recently, a promising cytotoxic activity with the essential oil against triple-negative breast tumor cells was undertaken. In this study was observed an important role in the discovery of novel therapeutics for the treatment of estrogen-negative tumors, which are more aggressive and not responsive to hormonal treatments (FURTADO, p.64, 2018)

On the other hands, we highlight the study of the hydroalcoholic extract and sesquiterpene lactone isolated from the same extract denominated 13-HDS (13-hydroxy-8,9-dehydro-shizukanolida). This compound produced a pronounced dose-dependent analgesic effect on abdominal constriction induced by intraperitoneal injection of acetic acid in rats, the latter being more effective than aspirin, acetaminophen and dipyrone, but less effective than morphine.

On the other hands, it should be noted that enzymes are molecules of proteins capable of modifying biochemical reactions, that is, they are catalysts. These processes occur due to factors such as temperature and pH. These factors are capable of altering the activity of the enzymes and, consequently, the speed of the reactions catalyzed by them. In this sense, each enzyme has an optimum pH of activity, in which its activity reaches the maximum peak. For most enzymes this pH is between 4.5 and 8.0. On the other hand, there is a direct relationship between the temperature and the speed of the enzymatic reactions, that is, the speed of the enzymatic reactions increases with the temperature until it reaches the maximum speed. Note that after reaching this peak the reaction speed begins to decrease, leading to enzymatic inhibition. According to Gareth (2003), these molecules are obvious targets for the design of drugs since the inhibition provides a method for preventing or regulating cell growth (GARETH, p. 190, 2003). In this regard,

Thus, in the present study, we have carried out antibacterial and enzymatic assays to available the biological potential of the essential oil of the *Hedyosmum brasiliense*
Mart. In addition, enzymatic inhibitory activity assays were performed to analyze the efficacy of the essential oil extracted from the *Hedyosmum brasiliense* Mart. against acetylcholinesterase and alpha-glucosidase enzymes.

### MATERIAL AND METHODS

To collect the plant material we chose the trail with approximately 100 meters of altitude, located inside the Mata Atlântica Park Hotel, an area protected by the Saint-Hilaire/Lange National Park, Paraná, Brazil (25° 67'17"S 48° 58' 29" W). The collection of the leaves of *H. brasiliense* was collected under the authorization of the System of Authorization and Information on Biodiversity - SISBIO number 49770-2. Also, information about the species was registered in the National System of Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN) under number A216E5A.

### EXTRACTION OF ESSENTIAL OIL

In order to collect the plant material, we decided to follow the trail with approximately 100 meters of altitude, located inside the Atlantic Rainforest Park Hotel, an area protected by the Saint-Hilaire / Lange National Park, Paraná, Brazil. After the collection, the samples were sent to the Laboratory of Sciences and Anato-Morphology of the Federal University of Paraná-Setor Litoral in Matinhos-PR to perform the process of oil extraction.

During the extraction procedure, the collected material was subjected to still fresh hydrodistillation. For the hydrodistillation process, 100 g of the high-precision electronic model material AD33OS and placed in a 2000 mL flask were weighed, followed by addition of 1000 mL distilled water.

During the procedure, a glass-shaped condenser (15cm) was used, which was isolated with aluminum foil to prevent light from coming in contact with the extracted material and for the operation of the cooling system. In this way, triplicates (R1, R2, R3) were carried out at maximum temperature until the boil was reached, reducing the temperature of the heating mantle later. The hydrodistillation period was approximately 4 hours (the time may vary according to the species in question).

The oils were collected with a 1000 μL pipette, then stored in a 1.5 ml numbered tube eppendorf and finally cooled in a vertical freezer to ensure the integrity of the essential oil. For the drying of the material was used centrifuge and micropipette of 200 μL. In addition, after extractions of the leaves of *Hedyosmum brasiliense* Mart. and stored in amber glass to perform the tests. The volatile oil was kept out of light reach and free of insoluble impurities.
in the Laboratory of Sciences and Anato-Morphology of the Federal University of Paraná - Setor Litoral in Matinhos-PR.

**ANTIBACTERIAL TESTS**

The antibacterial tests were carried out in the Chemical and Biological Pre-analysis laboratory of the UFPR (Federal University of Paraná - Setor Litoral) in Matinhos-PR.

Becker flasks were used with 1000mL of distilled H 2 O to perform the media with the following composition: LB culture medium: yeast extract 5g / L, sodium chloride 10g / L and tryptone 10g / L; LA culture medium: yeast extract 5g / L, sodium chloride 10g / L, tryptone 10g / L and 15g / L agar.

Sterilization was performed by vertical autoclaving at 100 ° C for 20 minutes.

The bacteria used for the study are the *Escherichia coli* strain NCM3722, which is associated with extra-intestinal infections and the *Staphylococcus aureus* strain ATCC 25923 which can provoke from simple to more severe skin infections. The sowing of the bacteria for antibacterial assay was performed in laminar flow hood model CFLH-09M to avoid contamination of the materials used. 10 ml of the LB medium was placed in a glass vial with 10 μl of the bacteria in 10% glycerol. Thus, the samples were precultured in a vertical incubator, at 37º C in an aerobic system, using a magnetic stirrer model 752A for 24 hours.

To perform the plates the solid medium was melted in an electric oven model CMW30ABANA for 5 minutes. Subsequently, 100 mL of LA medium was placed in the glass plate (9cm / 2cm) to solidify.

For the antibiotic solution, 980 were pipetted into 24 μL of distilled H 2 O and 20 μL of *Tetracycline* (C 22 H 24 N 2 O 8). According to Howland & Mycek (2007), “*tetracyclines* are effective against gram-positive and gram-negative bacteria as well as against other microorganisms other than bacteria (HOWLAND & MYCEK, p. 368, 2007)”.

Dilution of 2mL of the inoculated bacteria in 8mL of LB medium was carried out, using only 100μL in the plate and spread with a plastic handle (21.5cm / 3cm). Subsequently, three holes were drilled in the culture medium solidified with a sterilized pasteur pipette (150 mm), and 60 μL of the mineral oil (negative control) was pipetted in the first hole in the second 60 μL of the essential oil of *Hedyosmum brasiliense* Mart. and in the third 60 μL of the antibiotic (positive control). Finally, they were incubated at 37 ° C for 24 hours. After the 24-hour period the *Escherichia coli* and *Staphylococcus aureus* samples were analyzed and the medium used for the antibacterial assay was discarded.
ENZYME INHIBITION ASSAYS

The enzyme inhibition assays of the essential oils are carried out at the Natural Products Research Laboratory, located on campus 3, at the Department of Pharmaceutical Sciences of Regional University of Blumenau- Santa Catarina- Brazil. Reagents were obtained from SIGMA-Aldrich Saint Louis (MO-USA) and used without prior purification.

EVALUATION OF ACETYLCOLINESTERASE ACTIVITY

The acetylcholinesterase activity is determined in vitro by the spectroscopic method of Ellman et al. (1961). Samples are diluted in methanol at a concentration of 1 mg mL-1. 325 μl of Tris-HCl Buffer is added to maintain the pH of the medium in approximately 8. An aliquot of 100 μl of sample and 20 μl of acetylcholinesterase enzyme solution diluted in Tris-HCl buffer is added at 0, 1% Bovine Albumin Serum (0.25 U mL-1), this mixture being incubated at room temperature for 15 minutes. Then, 70 μL of acetylcholine iodide solution (0.021 mg mL-1), and 470 μL of Ellman’s Reagent (5,5-dithiobis (2-Nitrobenzoic acid), prepared in Tris HCl buffer added with NaCl 0 , 1M and 0.02 M MgCl2 · 6H2 O). After homogenisation, the test tubes are incubated under light for 25 minutes. Afterwards, 1000 μL of Tris-HCl buffer solution is added and the absorbance of the solution is measured in a spectrophotometer at a wavelength of 405 nm. As a positive control, a solution of Neostigmine hydrochloride (100 μg mL-1) is used, and as a negative control only the solvent used to dilute the samples. The inhibitory activity of the enzyme acetylcholinesterase is calculated by the equation:

Inhibitory Activity (%) =

\[ \frac{100 \cdot (\text{Abs sample} - \text{Abs blank}) \cdot 100}{\text{Average Abs do C. Negative}} \]

ACTIVITY EVALUATION A-GLUCOSIDASE

The α-glucosidase inhibition assay is performed as described by Kim et al. (2004). Sample solutions are prepared in methanol at a concentration of 1 mg mL⁻¹. At the time of testing, each solution is diluted with potassium phosphate buffer (pH 6.8) to 500 μg mL⁻¹. An aliquot of 50 μl of alpha-glucosidase solution (1 U mL⁻¹) was pre-mixed with 20 μL of the sample solutions, and 570 μL of potassium phosphate buffer (pH 6.8) (0.1 mol L⁻¹). All tubes were vortexed and incubated in a water bath at 37.5 °C for 20 minutes. After, 100 μl of p-nitrophenyl-α-D-glucopyranoside (pNPG, 1 mmol L⁻¹) is added as substrate and the reaction will start. The tubes are agitated again and the mixture is incubated for 30 min in a water bath at 37.5 °C followed by the addition of 650 μl of 1 M Na₂CO₃ solution to the completion
of the reaction. The amount of p-nitrophenol formed is measured in a spectrophotometer at wavelength of 410 nm for the estimation of the enzymatic activity. A 50 μg mL⁻¹ solution of Acarbose is used as standard. A negative control using only solvent, in place of the sample, is the one used. For each sample, a blank test is performed, where 20 μL of the sample solution (500 μg mL⁻¹) is added to 570 μL of potassium phosphate buffer (pH 6.8) (0.1 mol L⁻¹). The inhibitory activity of the enzyme alpha-glucosidase is calculated by the equation.

Inhibitory Activity (%) = \( \frac{\text{Abs C. Neg.} - (\text{Abs sample} - \text{Abs white}) \times 100}{\text{Abs C. Neg}} \)

### RESULTS AND DISCUSSION

#### Identification of Chemical Constituents

For the present study of the volatile components of the oil were considered only the compounds that were present in the oils in a percentage equal to or greater than 1%.

After the analysis of the essential oil from the leaves of the species, 14 compounds were identified; the others were inferior to the established value (≥1%). Table 1 below shows the chemical constituents of the essential oil of the species *Hedyosmum brasiliense* Mart.

<table>
<thead>
<tr>
<th>CONSTITUENTS</th>
<th>IA THEORETICAL*</th>
<th>IA*</th>
<th>KI*</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabinene</td>
<td>969</td>
<td>975</td>
<td>975</td>
<td>5.54</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>974</td>
<td>978</td>
<td>979</td>
<td>1.76</td>
</tr>
<tr>
<td>Cineole-1,8</td>
<td>1026</td>
<td>1033</td>
<td>1031</td>
<td>3.07</td>
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<tr>
<td>Pinocarvone</td>
<td>1160</td>
<td>1165</td>
<td>1164</td>
<td>1.97</td>
</tr>
<tr>
<td>Terpinel-4-ol</td>
<td>1174</td>
<td>1180</td>
<td>1177</td>
<td>1.36</td>
</tr>
<tr>
<td>β-Elemene</td>
<td>1389</td>
<td>1395</td>
<td>1390</td>
<td>1.35</td>
</tr>
<tr>
<td>γ-Muurolene</td>
<td>1478</td>
<td>1484</td>
<td>1479</td>
<td>4.11</td>
</tr>
<tr>
<td>Biciclogermacrene</td>
<td>1500</td>
<td>1501</td>
<td>1500</td>
<td>50.63</td>
</tr>
<tr>
<td>Cadinene</td>
<td>1522</td>
<td>1528</td>
<td>1523</td>
<td>1.31</td>
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<tr>
<td>Germacrene B</td>
<td>1559</td>
<td>1561</td>
<td>1561</td>
<td>2.15</td>
</tr>
<tr>
<td>Spathulenol</td>
<td>1577</td>
<td>1581</td>
<td>1578</td>
<td>2.68</td>
</tr>
<tr>
<td>Globulol</td>
<td>1590</td>
<td>1588</td>
<td>1590</td>
<td>1.16</td>
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<tr>
<td>Carotol</td>
<td>1594</td>
<td>1601</td>
<td>1594</td>
<td>19.06</td>
</tr>
<tr>
<td>Ferula lactone I</td>
<td>1974</td>
<td>1998</td>
<td>1974</td>
<td>1.90</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Monoterpenes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Oxygenated Monoterpenes</strong></td>
<td></td>
<td>7.44</td>
<td>6.52</td>
<td>60.75</td>
</tr>
<tr>
<td><strong>Sesquiterpenes</strong></td>
<td></td>
<td></td>
<td></td>
<td>25.29</td>
</tr>
<tr>
<td><strong>Oxygenated Sesquiterpenes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total identified % 98.05

*IA: Calculated Retention; IA teórico: Literature Retention Index; KI: Kovats Index.
*Index Aritimetical *Index Teorical Aritimetical.

Source: authors, 2019.
Bicyclogermacrene is the main constituent of essential oil extracted, with an average concentration of 50.63%. The next most abundant component was Carotol with a concentration of 19.06%. Essential oil coming from the leaves still indicated the presence of Sabinene (5.54%) and Muurolene (4.11%) as main components, besides 1,8-Cineole with 3.07%. In addition, a higher concentration of sesquiterpenes (60.75%) and oxygenated sesquiterpenes (25.29%) were verified in this study. Meanwhile, monoterpenes (7.44%) and oxygenated monoterpenes (6.52%) were identified in a lower concentration.

In this respect, it is important to note that the chemical composition may vary according to locality, climatic conditions and anthropic actions. In the other hands, Lima, Kaplan & e Cruz (2003) emphasize that the “identification of chemo-types should be considered an important item for quality maintenance, crop planning and phytopharmaceutical procurement (LIMA, KAPLAN & CRUZ, p.75, 2003)”. Finally, it should be noted that the combination of essential oil constituents may be effective against different etiological agents.

ASSAY WITH ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS BACTERIA

Antibacterial assays against *E. coli* strain oil (strain NCM3722) and *S. aureus* (ATCC 25923) were performed (table 2).

<table>
<thead>
<tr>
<th>BACTERIUM</th>
<th>AT</th>
<th>MO</th>
<th>EOHB</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>2,2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>3,0</td>
<td>0</td>
<td>1,2</td>
</tr>
</tbody>
</table>

Subtitle: **AT**= Antibiotic *Tetracycline* (positive control); **MO**= Mineral Oil (negative control); **EOHB**= Essential Oil *Hedyosmum brasiliense* Mart.

**Figure 2.** below shows the inhibition of the antibiotic *Tetracycline* (positive control) whereas for mineral oil (negative control) and essential oil (*Hedyosmum brasiliense* Mart.) There is no inhibitory halo.
Therefore, the essential oil coming from *Hedyosmum brasiliense* Mart. showed no activity against Gram-negative *Escherichia coli* bacteria.

The plaque tested against Gram-positive bacteria showed inhibition halo after established incubation period according to figure 3.

**Figure 2.** Plate after the 24-hour incubation period (without inhibition halo).

**Figure 3.** Plate after the 24-hour incubation period (with the presence of inhibition halo).
**Figure 3.** below shows the inhibition results of essential oils in millimeters from the standard used - the antibiotic “Tetracycline”.

**Figure 3.** Inhibition of the species *Hedyosmum brasiliense* Mart. in mm against *E. coli* and *S. aureus* bacteria

![Inhibition Results Diagram](image)

Subtitle: *E. coli* = *Escherichia coli*; *T1* = Tetracycline in the *E. coli* assay; *S. aureus* = *Staphylococcus aureus*; *T2* = Tetracycline in the *S. aureus* assay

Source: authors, 2019.

It was verified in this step the research that the oil of leaves from the *Hedyosmum Brasiliense* Mart. species harvested in the National Park Saint-Hilaire / Lange on the coast of Paraná do not have bacterial activity against *E. coli* strain (strain NCM3722). However, the oil coming from the species is selective to the *S. aureus* bacterium (strain ATCC 25923).

The result of the microbiological test of this research was distinct from the research carried out by Vido (2009), which used essential oil from *Hedyosmum brasiliense* Mart. of two locations in the state of São Paulo (Pindamonhangaba and Paranapiacaba). Vido (2009) achieved effective results against the bacteria tested (*Escherichia coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 8538)) using Dextrose Agar medium and antibiotic Chloramphenicol. It is noteworthy that “essential oils were more effective against Gram-positive bacteria than Gram-negative bacteria, results similar to most investigations of the antimicrobial potential of volatiles (VIDO, p.57, 2009)”. This result is related to the fact that gram-positive microorganisms have cell walls more prone to passage, whereas gram-negative microorganisms are more difficult to access due to cell membrane thickness.

Vido (2009) further states in his study that “against *Staphylococcus aureus*, most inhibitions of leaf oils collected at both sites were 100% (VIDO, p.57, 2009).” In addition, he adds that against *Escherichia coli* “the percentage of inhibition reached 100% in the spring (with most responses above 90%) (VIDO, p.57, 2009)”. 
Kirchner et al. (2009) in their study carried out the *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) assay, prepared in 1 ml of Mueller-Hinton medium agar (Difco). It was observed that the oil of *Hedyosmum brasiliense* showed activity against Gram-positive *S. aureus* bacteria (vol%: 0.312), whereas no activity against Gram-negative *E. coli* bacteria was identified, until the highest concentration used (2, 5%).

Luchesi (2017) performed a test with the essential oil coming from the species on Mueller Hinton agar and with the microorganisms *Staphylococcus aureus* INCQS 00015, *Escherichia coli* INCQS 00033, *Pseudomonas aeruginosa* INCQS 00025 and *Salmonella enteritidis* INCQS 00035. According to Luchesi (2017), the oil from *Hedyosmum brasiliense*, showed no activity against the bacteria at any concentration tested, this can be justified by volatilization of some compound, degradation or even oxidation, of mainly monoterpenic actives, although in its composition it presents 31.6% of Germacrene-B, 19.59% of Sabineno, 5.44% of α-Pinene, 7.23% of Eucalyptol, 6.41% of Carotol and other compounds that total the oil (LUCHESI, p.51, 2017).

It can be observed that several factors influence the obtaining of a positive result of antimicrobial action, such as volatilization, oxidation or even degradation of some component of the oil, facts that may have influenced this non-positive result.

### RESULTS OF ENZYME INHIBITION

The results of the in vitro inhibition assay of this study can be seen in Table 3 below.

<table>
<thead>
<tr>
<th>ENZYME</th>
<th>A (%)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholinesterase</td>
<td>69,82</td>
<td>3,46</td>
</tr>
<tr>
<td>α-glucosidase</td>
<td>5,15</td>
<td>0,40</td>
</tr>
</tbody>
</table>

Subtitle: A = Average; SD = Standard Deviation. Source: authors, 2019.

Figure 4. reveals the action of *Hedyosmum brasiliense* Mart. against AChE and alpha-glucosidase enzymes.
In this aspect, with the positive result obtained, the species can be considered as a potential anticholinesteric agent. Vido (2009) states that in his study in Paranapiacaba and Pindamonhangaba, “Fresh leaf oils collected at both sites were not effective in inhibiting acetylcholinesterase (VIDO, p. 61, 2009)”, which may be explained due to the fact that the composition of the majorities is distinct from that of this research. In addition, observing the result of inhibition, one can consider the oil coming from the species in function of the seasons of the year little effective against the enzyme alpha-glucosidase.

**CONCLUSION**

It was identified different active components present in the leaves of the species, being *Biciclogermacrene* the main constituent of the oil extracted, with a concentration of 50.63%. The *Carotol* constituent is the second most visible with concentration of 19.06%. In the analyzed sample there was a higher concentration of hydrocarbons sesquiterpenes (60.75%).

Subsequently, the antibacterial tests showed a non-significant result for the oil coming from the four seasons of the year against the strain of *E. coli* (NCM3722). However, the oil was selective for the *S. aureus* bacterium (ATCC 25923), with a significant result with a 12mm inhibition halo.

In addition, in the enzyme inhibition assay against the acetylcholinesterase enzyme, a percentage of inhibition of 69.82% was found. In this respect, observing the positive result for inhibition of AChE, the species can be considered as a potential anticholinesterase agent. However, the inhibition test against the alpha-glucosidase enzyme did not show such
an effective result varying at 5.15%. Finally, it should be noted that the results of studies of biological activity of the species *Hedyosmum brasiliense* Mart. are promising in order to validate and recognize their potential.

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