

## Chemical Profile, Toxicity, Anti-Acetylcholinesterase and Anti-microbial Activity of Essential Oil from *Hyptis dilatata* Leaves

Sirley P. Almeida<sup>\*a</sup>, Antonio Alves de M. Filho<sup>b</sup>, Fernanda Guilhom Simplicio<sup>c</sup>, Regildo M. G. Martins<sup>d</sup>, Jacqueline A. Takahashi<sup>e</sup>, Vany P. Ferraz<sup>f</sup>, Ana Cristina G. R. de Melo<sup>g</sup>, Wanderli P. Tadei<sup>h</sup>

<sup>a</sup>Post graduate in Biodiversity and Biotechnology Program, PPG BIONORTE, State Coordination of Amazonas, Manaus, Amazonas, Brazil.

<sup>b</sup>Postgraduate Program in Chemistry, PPGQ, Center for Research and Graduate Studies in Science and Technology, NPPGCT, Department of Chemistry and Postgraduate in Biotechnology and Biodiversity Program, Rede Bionorte, Federal University of Roraima, Paricarana Campus, CEP 69304-000, Boa Vista-RR-Brazil.

<sup>c</sup>Laboratory of Phytochemistry and Semi synthesis, Faculty of Pharmaceutical Sciences, Federal University of Amazonas, CEP 69077-000, Manaus, Amazonas, Brazil.

<sup>d</sup>Federal University of Pernambuco, Immuno Pathology Laboratory Keizo-Ami, Recife-PE- Brazil.

<sup>e</sup>Department of Chemistry, Institute of Exact Sciences, Universidade Federal de Minas Gerais, CEP 31270-901, Belo Horizonte, Minas Gerais, Brazil.

<sup>f</sup>Chromatography Laboratory, Institute of Exact Sciences, Federal University of Minas Gerais, CEP 31270-901, Belo Horizonte, Minas Gerais, Brazil.

<sup>g</sup>Post graduate in Natural Resources Program, Federal University of Roraima, Boa Vista-RR-Brazil.

<sup>h</sup>National Institute for Amazonian Research, CEP 69067-375, Manaus, Amazonas, Brazil.

[silacarneiro@bol.com.br](mailto:silacarneiro@bol.com.br)

The aim of this study was to evaluate the influence of seasonality on the chemical composition of the essential oil from *Hyptis dilatata* leaves, to perform biological activity assays such as antimicrobial, inhibition of acetylcholinesterase enzyme and to evaluate the toxicity of the essential oil using *Artemia salina* as indicator on the test. *Hyptis dilatata* leaves were collected in rainy and dry seasons, in the morning, in the afternoon and in the evening. It was extracted by hydrodistillation using the extractor of Clevenger condenser double Spell model. The essential oil analysis resulted in 22 chemical components. The major constituents of the dry and rainy periods were  $\alpha$ -pinene (18.7% in the night and 12.9% in the afternoon), 3-carene (26.5% and 19.9% in the night), fenchone (43% and 33.7% in the morning) and  $\beta$ -caryophyllene 9.1% in the afternoon and 6.1% in the morning. The essential oil *in vitro* inhibited the acetylcholinesterase enzyme in 99.9% in the afternoon (rainy period) and 96.4% in the morning (dry period). Between the dry and rainy seasons, the lowest LC<sub>50</sub> microbial activity *in vitro* was obtained for leaves collected during rainy season, in the morning period tested against the bacterium *Staphylococcus aureus* (LC<sub>50</sub> 78.1 mg mL<sup>-1</sup>). The cytotoxic activity of the essential oil of *H. dilatata* on *Artemia salina* showed LC<sub>50</sub> results below 100  $\mu$ g mL<sup>-1</sup> and in the afternoon during the rainy period and at night in the dry period the results are above this value. Therefore, the chemical characterization and biological activities tests of essential oils showed promising results in the search for new active substances and the development of bioproducts of vegetable origin.

### 1. Introduction

The genus *Hyptis* belongs to the Lamiaceae family that is formed by herbs and shrubs, which are composed of approximately 580 species distributed mainly in tropical America, from the south of the United States to Argentina (Lima, 2010). In Brazil, it is mainly found in the states of Minas Gerais, Bahia, Goiás and Amazonas. It is a genus in species of great ethnopharmacological importance, since populations used it for medicinal purposes, not only in Brazil, but also in Mexico, Colombia, Panama and other places (Falcão and Menezes, 2003). Costa (2013) reports that species of this genus have a very variable chemical constitution, showing cytotoxic, antimalarial, antimicrobial, expectorant and antiviral activities.

We believe that better understanding the impact of those aspects are of the utmost importance for the developing of technologies to improve the pharmacological potential of this medicinal species, focused mainly on its phytotherapeutic application, once this quality of essential oils are linked to their chemical constitution (Martins et al., 2006). The chemical composition of plants of this species, found in different localities, may present variations depending on the type of soil, temperature, climate and altitude (Gobbo-Neto and Lopes, 2007; Miranda et al., 2016).

According to Morais (2009), the metabolic activities of essential oils can be influenced by stimulus of the environment, in which the plant is found, leading to the synthesis of different compounds. The plant under study in this work is the *H. dilatata* Benth species, belonging to the Lamiaceae family. Essential oil research involves the seasonal period (dry and rainy) to evaluate the presence and/or qualitative changes of the chemical compounds of the essential oils, in each period and time (morning, afternoon and night).

The interest in this plant resulted from being of mountainous area, collected in Tepequém Mountains in Amajari municipality in Roraima state, Brazil. The collection occurred in the dry period and in the rainy period. In this work, we evaluated samples collected in different seasonal periods and correlated them to the chemical compounds of their essential oils. We tested their antimicrobial, fungicide, insecticide activities and inhibiting of the enzyme acetylcholinesterase, as well as antioxidant and toxic activity against *Artemia salina*.

## 2. Material and Methods

### 2.1 Botanical Identification and essential oil extraction

The plant material of *H. dilatata* was collected in August 2014, in Paiva village, on the margin of RR 203, in Tepequém Mountains, Amajari municipality, Roraima, Brazil. The mountain location where *H. dilatata* leaves were collected is at 634 m (meters above sea level). The voucher specimen was deposited in the INPA herbarium with registration number 263,670. The other voucher specimen sample was deposited in the integrated museum of Roraima, MIR 12754. Authorization for collection is SISBIO 44983-2. The extraction of the essential oil from the fresh leaves collected in the morning, afternoon and night hours were realized in triplicate and were coupled to the Clevenger apparatus, initiating the extraction of the volatile oil using the hydrodistillation method, for two hours. Table 1 shows the weight of leaves for essential oil extraction, collected during the rainy and dry periods.

Table 1: weight of leaves collected in the rainy and dry periods

|           | Rainy    | Dry      |
|-----------|----------|----------|
| Morning   | 270.15 g | 575.26 g |
| Afternoon | 517.87 g | 507.53 g |
| Night     | 632.42 g | 763.08 g |

### 2.2 CGFID analysis

The majority of chemical constituents present in the essential oils were determined using a CGFID-HP7820A (Agilent). Column: HP5 30 m × 0.32 mm × 0.25 μm (Agilent). Temperature: Column: 50°C (0 minutes), 0°C min<sup>-1</sup>, up to 230°C. Injector: 250°C Split (1:30). Detector FID: 250°C. Vector gas: H<sub>2</sub> at 3.0 mL min<sup>-1</sup>. Injection volume: 1 μL. Data acquisition software: EZChrom Elite Compact (Agilent). Samples diluted at 1% in chloroform.

### 2.3 Antiacetylcholinesterase activity of essential oil from leaves of *H. dilatata*

Quantitative evaluation of acetylcholinesterase (AChE) inhibition activity was performed according to the methodology of Ellman (1961), modified by Rhee et al. (2001). This bioassay was performed on microplates of 96 wells. Eserine and galantamine (10 mg mL<sup>-1</sup>) were used as positive controls while the negative control was performed without inhibitor. In each well were added 25 μL of acetylcholine iodide (15 mM); 125 μL of 5.5'-dithiobis (2-nitro benzoic acid) (DTNB); 50 μL of tris-HCl pH 8 0.1% w/v buffer of bovine serum albumin and 25 μL of extract (10 mg mL<sup>-1</sup>) solubilized in Tween/DMSO (30:70). The tests were performed in triplicate. The plates were read nine times at 405 nm over a period of 10 minutes. Immediately after the first reading, 25 μL of acetylcholinesterase enzyme (*Electrophorus electricus*, Sigma Aldrich) (0.222 U mL<sup>-1</sup>) was added and nine readings were performed over a period of 10 minutes at 405 nm. The interference of spontaneous hydrolysis

of the substrate was corrected from the subtraction of the average of the absorbance measured before enzyme addition. The enzyme inhibition percentage was calculated from the following mathematical formula:

$$\% \text{ inhibition} = [(C - A) / C \times 100]$$

where C = control containing enzyme and substrate; A = assay containing the extract, enzyme and substrate. The data obtained were treated using Microsoft Origin 6.1 software.

#### **2.4 Toxicity determinations of *H. dilatata* leaves essential oil on *Artemia salina***

The toxicity tests on *A. salina* were carried out using the methodology adapted from the work of (Meyer et al. 1982). An artificial saline solution (40g of coarse salt in 1L of distilled water) was added in an aquarium which was used as an incubator, adjusting the pH between 8 and 9 with a solution of sodium carbonate ( $\text{Na}_2\text{CO}_3$  10%). After hatching, 10 specimens (*A. salina* nauplii) were selected and exposed to the different extracts and essential oil within test tubes with the following concentrations: 1000; 500; 250; 125; 62.5; 31.25 and 15.62  $\mu\text{L mL}^{-1}$ . The tests were performed in triplicate, for each concentration. A saline solution without extract and another tube with DMSO were used as positive control. This system was incubated at room temperature for 24 hours without aeration and the tubes were kept under illumination. After an incubation period of 24 hours they were verified and the number of live and dead larvae in each tube was counted, through macroscopic visualization.

#### **2.5 Determinations of the antimicrobial and fungicidal activity of the *H. dilatata* obtained from leaves.**

In order to verify the antimicrobial activity of the essential oils of leaves of *H. dilatata*, pathogenic microorganisms such as *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), Gram-negative *Salmonella typhimurium* (ATCC 13311), *Citrobacter freundii* (ATCC 8090) and Yeast: *Candida albicans* (ATCC 18804) were used, in which concentrations were: 250; 125; 62.5; 31.25; 15.6; 7.8; 3.9 and 1.95  $\mu\text{g mL}^{-1}$  (ZACCHINO;GUPTA, 2007).

The samples were weighed to 0.0125 mg and dissolved in 1 mL of dimethyl sulfoxide (DMSO) resulting in a concentration of 12.5  $\text{mg mL}^{-1}$  to obtain the essential oil. Then 124  $\mu\text{L}$  of this solution was added to a vial of eppendorf containing 2976  $\mu\text{L}$  of BHI (Brain Heart Infusion) broth for bacteria and 2976  $\mu\text{L}$  of Sabouraud for yeast. Subsequently, a pre-inoculum was prepared, in which the bacteria and yeast stored under refrigeration were transferred with a platinum ring to test tubes containing 3 mL BHI broth.

The tests were performed on 96 microwells Elisa plates in triplicate. In each well was added 100  $\mu\text{L}$  of the BHI culture medium. In well 1 were also inserted 100  $\mu\text{L}$  of the working solution. The solution was homogenized and 100  $\mu\text{L}$  were transferred to the next well consecutively. The 100  $\mu\text{L}$  final were discarded for each sample, then 100  $\mu\text{L}$  of the microorganism suspension were added to each well. Two controls were used, one to monitor the growth of the microorganism growth, in which there was no addition of the working solution and a blank, in which the bacterial inoculum was not added (to eliminate any coloring effect of the working solution). A control plate, containing 100  $\mu\text{L}$  of BHI culture medium and 100  $\mu\text{L}$  of sterile distilled water, was added to the experiment to control the sterility of the BHI culture medium sterility. Another control was prepared containing the standard antibiotics: Ampicillin (antibacterial), Miconazole and Nystatin (antifungals) to observe the activity of these antibiotics on microorganisms. The microplates were incubated in an oven at 37 °C and after 24 hours the Elisa plates were read (492 nm). The results were calculated as percent inhibition using this formula:

$$\% \text{ Inhibition} = 100 - AC1 - AC2 \times 100AH-AM$$

AC1 = Sample absorbance; AC2 = Sample control absorbance; AH = absorbance in the control of microorganism and AM = absorbance of the control of the culture medium.

### **3. Results and discussion**

#### **3.1 GC-FID analysis**

Analysis of GC-FID for quantification of the chemical components of the essential oil (OE) of the leaves of *H. dilatata* showed 22 constituents present in the oils extracted from the leaf, obtained in the dry and rainy seasons at different times (morning, afternoon and night). The major constituents are shown in Table 2.

Table 2: Constituents Percentages identified in essential oils of *Hyptis dilatata* leaves

| Peak             | Compounds                     | *KI  | *RT min | DML (%) | RML(%)<br>DAL(%) | RAL(%) | DNL(%) | RNL(%) |      |
|------------------|-------------------------------|------|---------|---------|------------------|--------|--------|--------|------|
| 1                | $\alpha$ -pinene              | 973  | 3.75    | 10.0    | 10.2             | 5.8    | 12.9   | 18.7   | 11.9 |
| 2                | Camphene                      | 980  | 4.03    | 0.8     | 0.6              | 0.5    | 0.7    | 1.1    | 0.6  |
| 3                | Sabinene                      | 993  | 4.53    | 0.0     | 0.0              | 0.1    | 0.1    | 0.0    | 0.1  |
| 4                | $\beta$ -pinene               | 996  | 4.65    | 2.0     | 2.5              | 1.5    | 2.9    | 2.9    | 3.1  |
| 5                | Myrcene                       | 1008 | 5.07    | 0.8     | 0.7              | 0.8    | 0.8    | 1.3    | 0.9  |
| 6                | $\alpha$ -felandrene          | 1015 | 5.37    | 0.2     | 0.2              | 0.3    | 0.2    | 0.3    | 0.2  |
| 7                | 3-carene                      | 1019 | 5.52    | 16.2    | 15.1             | 13.6   | 18.6   | 26.5   | 19.9 |
| 8                | $\alpha$ -terpinene           | 1024 | 5.70    | 0.2     | 0.2              | 0.2    | 0.2    | 0.3    | 0.2  |
| 9                | p-cymene                      | 1031 | 5.94    | 0.3     | 0.3              | 0.3    | 0.3    | 0.4    | 0.5  |
| 10               | Limonene                      | 1033 | 6.04    | 3.5     | 2.6              | 2.8    | 2.9    | 4.9    | 3.2  |
| 11               | g-terpinene                   | 1058 | 6.96    | 0.5     | 0.4              | 0.5    | 0.4    | 0.7    | 0.5  |
| 12               | Fenchone                      | 1083 | 7.90    | 43.0    | 33.7             | 36.3   | 29.6   | 27.0   | 30.8 |
| 13               | Menthenol                     | 1110 | 8.95    | 0.6     | 0.6              | 0.4    | 0.8    | 0.5    | 0.7  |
| 14               | Camphor                       | 1133 | 9.80    | 3.1     | 2.6              | 3.0    | 2.1    | 1.7    | 2.1  |
| 15               | Fenchol                       | 1170 | 11.20   | 0.7     | 1.1              | 1.4    | 1.0    | 0.7    | 1.2  |
| 16               | Terpinen-4-ol                 | 1190 | 11.96   | 0.7     | 1.0              | 1.0    | 1.1    | 0.7    | 0.9  |
| 17               | $\alpha$ -terpineol           | 1212 | 12.77   | 0.1     | 0.1              | 0.2    | 0.0    | 0.0    | 0.0  |
| 18               | $\beta$ -caryophyllene        | 1413 | 20.39   | 4.6     | 6.1              | 9.1    | 5.8    | 4.5    | 4.2  |
| 19               | Aromadendrene                 | 1432 | 21.10   | 0.2     | 0.3              | 0.3    | 0.2    | 0.2    | 0.2  |
| 20               | Humulene                      | 1447 | 21.66   | 0.4     | 0.6              | 0.7    | 0.6    | 0.4    | 0.4  |
| 21               | D-germacrene<br>Caryophyllene | 1491 | 23.31   | 0.1     | 0.1              | 0.3    | 0.1    | 0.1    | 0.1  |
| 22               | Oxide                         | 1574 | 26.47   | 1.1     | 1.7              | 1.7    | 1.6    | 0.6    | 1.3  |
| Total identified |                               |      |         | 89.1    | 79               | 80.8   | 82.9   | 76.5   | 73   |
| Others           |                               |      |         | 10.9    | 21               | 19.2   | 17.1   | 23.5   | 27   |

\*DML - Dry morning leaves; RML- Rainy morning leaves; DAL- Dry afternoon leaves; RAL- Rainy afternoon leaves; DNL- Dry night leaves; RNL- Rainy night leaves. \*KI= Kovats index; \*RT= Retention time.

Among the constituents found in the chemical characterization of the essential oils of the leaves of *H. dilatata*, the major compounds were: fenchone, 3-carene,  $\alpha$ -pinene,  $\beta$ -caryophyllene, Limonene,  $\beta$ -pinene and camphor. Fenchone (1,3,3-trimethylbicyclo-[2,2,1]-heptan-2-one) was the chemical constituent with the highest concentration (43%) in the essential oils of the leaves collected in the morning at the dry period. The second chemical constituent with the highest concentration found in the essential oil of the leaf collected in the dry period at night was 3-carene (26.5%).

The chemical composition of the *H. dilatata* essential oils was mainly monoterpenes and sesquiterpenes. According to Rocha (2013), the environmental conditions can cause significant variations in the composition of plant oils. Comparing the constituents of the *H. dilatata* essential oil of the leaves collected in Tepequém Mountains in Roraima state with the same type of oil collected in Arauca, Colombia, by Tafurt-Garcia et al. (2014), it was observed that some constituents have the same concentration value, but differ when comparing the different samples of the plant collected in Roraima. In the plants collected in Arauca, Colombia, 3-Carene (11%), Limonene (4.9%),  $\beta$ -pinene (0.7%),  $\alpha$ -Pinene and fenchone (<0.05%) were described (Tafurt-Garcia et al., 2014).

Meanwhile, constituents of leaf essential oil collected in Tepequém Mountains in Roraima state obtained the following concentrations in relation to the same constituents of the essential oils found in the leaf collected in Colombia: 3-carene in dry period at night (26,5%), limonene in dry period at night (4.9%),  $\beta$ -pinene at night in the rainy period (3.1%),  $\alpha$ -pinene at night and in the dry period (18.7%) and fenchone at morning in the dry

period (43%). Studies in Colombia with the genus of *H. dilatata*, performed by Tafurt-Garcia et al. (2014), showed quantitative and qualitative differences in relation to the constituents in different species of plants from Lamiaceae family, also differentiating from the compounds found in the specimens studied in this work.

### 3.2 Inhibition activity of the enzyme acetylcholinesterase (AChE)

The inhibition of AChE promoted by the essential oils of the leaves collected in the rainy season in the afternoon was approximately 99.29%, while in the dry period, the highest inhibition occurred for the essential oils of the leaves collected in the dry period in the morning, 96.46%. The essential oil of *H. dilatata* from the leaves collected in the dry period on AChE showed inhibition levels of 79.9%, whereas it was lower for leaves collected in the rainy season (51.50%). This difference may be related to production of secondary metabolites in different periods. Vinutha et al. (2007) divided as: potent inhibitors - above 50%; moderate - between 30% and 50% and weak - below 30%. Therefore, the essential oil studied in this work presents potential inhibitory activity against the enzyme acetylcholinesterase.

### 3.3 *Artemia salina* Toxicity

In the toxicity tests performed with essential oils from *H. dilatata* leaves against *A. salina*, the death percentage nauplii was observed after 24 hours, allowing LC<sub>50</sub> calculation, showing the toxic potential of this species. The lowest LC<sub>50</sub> occurred with the essential oil extracted from the leaves collected in the morning in the rainy period, LC<sub>50</sub> = 23.4 µg mL<sup>-1</sup>, degree of reliability (10.9 - 33.5), the second most toxic was oil LC<sub>50</sub> = 44.3 (33.0 - 57.2). Table 3 shows the LC<sub>50</sub> values and the reliability of results for all tested oils.

Table 3: LC<sub>50</sub> values in the essential oils of *H. dilatata* against *A. salina* larvae in 24 hours reading

| Oils | LC <sub>50</sub> (CI 95%) | Slope ± SD  | χ <sup>2</sup> | DF |
|------|---------------------------|-------------|----------------|----|
| RML  | 23.4 (10.93 -37.53)       | 1.13 (0.19) | 2.14           | 5  |
| DML  | 76.3 (45.16 -121.99)      | 1.75 (0.21) | 8.17           | 5  |
| RAL  | 112.2 (73.85 -180.80)     | 1.08 (0.20) | 0.26           | 4  |
| DAL  | 69.5 (42.34- 103.95)      | 1.06 (0.17) | 4.75           | 5  |
| RNL  | 44.3 (33.08-57.20)        | 2.03 (0.26) | 3.34           | 5  |
| DNL  | 126.0 (83.81-189.08)      | 1.08 (0.16) | 1.89           | 5  |

\* RML - Rainy morning leaves; DML - Dry morning leaves; RAL- Rainy afternoon leaves; DAL - Dry afternoon Leave; RNL - Rainy night leaf; DNL - Dry night leave. \* CI - Confidence interval; \* SD - Standard deviation; \* DF - Degree of freedom.

According to Amarante (2011), LC<sub>50</sub> values for mortality calculations are: low toxicity when LC<sub>50</sub> is greater than 500 µg mL<sup>-1</sup>, moderate for LC<sub>50</sub> between 100 to 500 µg mL<sup>-1</sup> and very toxic when LC<sub>50</sub> was lower to 100 µg mL<sup>-1</sup>. In the negative controls, there was no mortality, and it was not necessary to apply the Abbott formula. The solvent used showed no interference in the results, therefore the activities presented are related to the samples tested. The essential oils of *H. dilatata* species studied in this work had toxicity to *A. salina* lower than 100 µg mL<sup>-1</sup>.

### 3.4 Antimicrobial and fungicidal activity of *H. dilatata* oil from leaves

The antibacterial and fungicidal potentials of the essential oils from *H. dilatata* were evaluated on Gram-positive bacteria: *Staphylococcus aureus*, *Bacillus cereus* and Gram-negative: *Salmonella typhimurium*, *Citrobacter freundii* and Yeast: *Candida albicans*. The inhibition values of leaf essential oils collected in the dry period, in the afternoon hour, showed, for bacteria, that the lowest IC<sub>50</sub> value the best inhibition towards the Gram positive bacterium *B. cereus* (IC<sub>50</sub> = 112.8 µg mL<sup>-1</sup>). Regarding the essential oils of the leaf collected during the rainy season, only Gram-positive bacteria were inhibited. The lowest inhibition occurred with leaf oil collected in the morning IC<sub>50</sub> = 78.8 µg mL<sup>-1</sup> against *S. aureus* bacteria. The control used in the bacterial assays was to penicillin IC<sub>50</sub><1.95 µg mL<sup>-1</sup>. The percentage of inhibition ranged from the highest concentration of 250 µg mL<sup>-1</sup> (94.0%) to the lowest concentration 1.95 µg mL<sup>-1</sup> (92.8%).

In the case of biological assays with the bacteria in this study, the essential oils of the leaves on the Gram-positive bacteria were more effective. Vaara (1992) explains that Gram negative bacteria show resistance to several types of antibiotics. This is due to an outer membrane that surrounds the cell wall, difficulting the transport of hydrophobic substances. The Carnosic acid already studied in species *H. dilatata* as reported by Urones et al. (1998) and Oluwatuyi et al. (2004) has activity against strains of *S. aureus*, which are quite resistant.

#### 4. Conclusions

Considering the potential of the essential oil extracted from the leaves, at different seasons, one can conclude that of the 22 chemical constituents identified in the chemical characterization of the essential oils of the leaves of *H. dilatata*, in different climatic periods such as dry and rainy, the majority were: fenchone, 3-carene,  $\alpha$ -pinene,  $\beta$ -caryophyllene, limonene,  $\beta$ -pinene and camphor. There was no difference in the type of constituents in the dry and rainy periods, only in relation to the percentage of their concentration. The results of biological activities with the acetylcholinesterase enzyme, bacterial assays, fungicides and cytotoxicity indicate that they may be related to the action of the chemical constituents presented in the chemical characterization of the essential oils collected in the dry and rainy periods at different times, in varying concentrations of the chemical compounds present.

#### Acknowledgement

To CNPq, FAPEMIG and FAPEAM for scholarships and financial support.

#### Reference

- Amarante C.B., Muller A.H., Póvoa M.M., Dolabela M.F., 2011, Phytochemical study biomonitoring by toxicity tests front of *Artemia salina* and antiplasmodial activity aninga stem (*Montrichardia linifera*). *Acta Amaz.* 41, 431 – 434.
- Costa V.C.D.O., 2013, Contribution to the chemical knowledge of *Hyptis* species occurring in the Paraíba semi-arid region: *Hyptis macrostrachys* Benth and *Hyptis umbrosa* Salzm. Thesis (PhD in Natural and Synthetic Bioactive Products) - Federal University of Paraíba, Health Sciences Center, João Pessoa-PB, 162p.
- Ellman G.L., 1961, A new and rapid colorimetric determination of acetylcholinesterase activity. *Bioch. Pharm.* 7, 88-95.
- Falcão D.Q., Menezes F.S., 2003, Ethnopharmacological, pharmacological and chemical revision of the genus *Hyptis*. *Rev. Bras. Farm. Rio de Janeiro*, 84, 69-7.
- Gobbo-Neto L., Lopes N.P., 2007, medicinal plants: factors influencing the content of secondary metabolites. *Chem. Nova.* 30, 374-81.
- Lima K.S.B., 2010, Contribution to the chemical knowledge of *Hyptis carvalhoi* Harley. Federal University of Ceará, Graduate Program in Chemistry. Fortaleza - CE, Brazil.
- Martins F.T., Santos M.H., Polo M., Barbosa L.C.A., 2006, Chemical variation of the essential oil of *Hyptis suaveolens* (L.) Poit, under cultivation conditions. *New Chem.* 29, 1203-1209.
- Meyer B.N., Ferrigni N.R., Putnam L.B., Jacobsen D.E., Nichols and McLaughlin J.L., (1982), A convenient general bioassay for active plant constituents. *Plant Méd.* 45, 31-34.
- Miranda C.A.S.F., Cardoso M.G., Batista L.R., Rodrigues L.M.A., Figueiredo A.C.S., 2016, Essential oils from leaves of various species: Antioxidant and antibacterial properties in growing pathogenic species. *Ciênc. Agron.* 47, 213-220.
- Morais L.A.S., 2009, Influence of abiotic factors on the chemical composition of essential oils. *Hort. Bras.* 27, 4050-4063.
- Oluwatuyi, Kaatz G.W. Gibbons S., 2004, antibacterial and resistance modifying activity of *Rosmarinus officinalis*. *Phyto chem. Oxford*, 65, 3249-3254.
- Rhee I. K. Van der Meent M., Ingkaninan K., Verpoorte R., 2001, Screening for acetylcholinesterase inhibitors from *Amararyllidaceae* using silica gel thin-layer chromatography in combination with bioactivity staining. *Journal of Chromatography A*, 915, 217-223.
- Rocha B.C.A., 2013, Extraction and characterization of thyme oil-*Thymus vulgaris*. Dissertation (Master degree in Chemical Engineering) - Federal Rural University of Rio de Janeiro, Soropédica, 150p.
- Tafurt-García G., Muñoz-Acevedo A., Calvo A.M., Jiménez L.F., Delgado W.A., 2014, Volatile components of *Eriope crassipes*, *Hyptis conferta*, *H. dilatata*, *H. brachiata*, *H. suaveolens* y *H. mutabilis* (Lamiaceae). *Latin American and Caribbean Bulletin of Med. and Aromatic Plants* 13, 254 – 269.
- Urones J.G., Marcos I.S., Diez D., Cubilla L.R., 1998, Tricyclic diterpenes from *Hyptis dilatata*. *Phytochem.* 48, 1035-1038,
- Vaara M., 1992, Agents that increase the permeability of outer membrane. *Microb. Reviews.* 56, 395-411.
- Vinutha B (2007). Screening of Selected Indian Medicinal Plants for Acetylcholinesterase Inhibitory Activity. *J. Ethnopharm.* 109(2): 359-363.
- Zacchino A.S., Gupta M.P., 2007, Manual of in vitro techniques for the detection of antifungal compounds. Corpus Editorial and Distributor: Rosario. 85.