

Chemical profile and larvicidal activity of essential oil obtained from the leaves of *Plectranthus amboinicus* (Lour.) Spreng

Perfil químico e atividade larvívica de óleo essencial obtido a partir das folhas de *Plectranthus amboinicus* (Lour.) Spreng

Perfil químico y actividad larvívica del aceite esencial obtenido de las hojas de *Plectranthus amboinicus* (Lour.) Spreng

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Abstract

This study aimed to determine the chemical constituents and larvicidal activity of the essential oil of *Plectranthus amboinicus* (Lour.) Spreng against larvae of the *Aedes aegypti* mosquito. The essential oil (EO) was extracted by hydrodistillation at 100 ° C for 3 hours. The chemical composition was obtained by Gas Chromatography coupled to Mass Spectrometry (GC / MS). To assess larvicidal activity, *Aedes aegypti* larvae were subjected to EO solutions in concentrations of 10-100 mg L⁻¹, where larval mortality was assessed and the LC₅₀ was determined using the Probit method. The main chemical constituent found in the EO was carvacrol, which is considered to be very promising for pharmaceutical synthesis. The EO showed larvicidal activity with an LC₅₀ of 28.52 mg L⁻¹. According to the results found, it was possible to evaluate that the analyzed EO is composed of substances that have an efficient larvicidal effect against *Aedes aegypti*, thus encouraging its potential for application.

Keywords: Essential oils; Larvicide; Chemical constituents.

Resumo

Este estudo teve por objetivo determinar os constituintes químicos e atividade larvídica do óleo essencial de *Plectranthus amboinicus* (Lour.) Spreng frente a larvas do mosquito *Aedes aegypti*. O óleo essencial (OE) foi extraído por hidrodestilação a 100 ° C por 3h. A composição química foi obtida por Cromatografia Gasosa acoplada à Espectrometria de Massas (GC/MS). Para avaliação da atividade larvídica submeteu-se larvas de *Aedes aegypti* a soluções do OE em concentrações de 10-100 mg L⁻¹, onde avaliou-se a mortalidade das larvas e determinou-se a LC₅₀ a partir do método de Probit. O principal constituinte químico encontrado no OE foi o carvacrol sendo considerado grande promissor para síntese farmacêutica. O OE apresentou atividade larvídica com LC₅₀ de 28,52 mg L⁻¹. De acordo com os resultados encontrados, foi possível avaliar que o OE analisado é composto por substâncias que possuem um eficiente efeito larvídica frente ao *Aedes aegypti*, incentivado assim seu potencial de aplicação.

Palavras-chave: Óleos essenciais; Larvídica; Constituintes químicos.

Resumen

Este estudio tenía como objetivo determinar los componentes químicos y la actividad larvídica del aceite esencial de *Plectranthus amboinicus* (Lour.) Spreng. Estorbo frente a las larvas del mosquito *Aedes aegypti*. El aceite esencial (AE) fue extraído por hidrodestilación a 100 ° C durante 3h. La composición química fue obtenida por cromatografía de gases acoplada a espectrometría de masas (CG/EM). Para evaluar la actividad larvídica, las larvas de *Aedes aegypti* se sometieron a soluciones de AE a concentraciones de 10-100 mg de L⁻¹, donde se evaluó la mortalidad por larvas y se determinó CL₅₀ a partir del método Probit. El principal componente químico encontrado en AE fue carvacrol siendo considerado un muy prometedor para la síntesis farmacéutica. El AE mostró actividad larvídica con CL₅₀ de 28,52 mg L⁻¹. Según los resultados encontrados, fue posible evaluar que el AE analizado está compuesto por sustancias que tienen un efecto larvídica eficiente contra *Aedes aegypti*, fomentando así su potencial de aplicación.

Palabras clave: Aceite esencial; Larvídica; Compuestos químicos.

1. Introduction

Plants are considered one of the main natural resources for medicinal use, due to their biological potential, whether due to the action of deadly diseases or diseases that affect living beings, therefore, according to the World Health Organization, almost 80% of the population in developing countries development uses them directly or indirectly for their basic health needs, either because of cultural tradition or because there are no other options, due to the high cost of traditional medicines for this population (Bermúdez, Oliveira-Miranda & Velázquez, 2005; Alitonou et al., 2012; de Souza et al., 2020).

Multidisciplinary efforts have also led to an increase in the number of researches to obtain greater knowledge about a medicinal plant (de Souza et al., 2020). The study of substances extracted from plants has been shown to be indispensable over time, whether due to the great biological diversity in Brazil or the potential of this extraction. Thus, essential oils represent a viable alternative in several studies involving substances of plant origin (Gomes et al., 2019; Minott & Brown, 2007).

Specific analyzes are necessary around the validation process, which includes chemical composition, proof of pharmacological, molluscicidal, microbial activity and possible toxicity in humans parameters that ensure the quality of raw materials of plant origin (Gomes et al., 2019).

The toxic potential of essential oils (EOs) and their compounds can vary significantly according to intrinsic and extrinsic factors (Kim et al., 2016). The chemical composition of EOs contributes significantly to the determination of the pharmacological potential attributed to plant species. In the case of EOs, it is recommended to regularly use the gas

chromatography system coupled to the mass spectrum (GC / MS), in order to identify substances derived from secondary metabolism. EO extracted from medicinal plants have been widely used successfully in research aimed at epidemiological control (Mirzahosseini et al., 2017).

According to the Ministry of Health (2019), it was declared that in 2019, until the 12th Epidemiological Week (12/30/2018 to 03/23/2019), there were 273,193 probable cases of dengue in the country, which had a growth of approximately 382% compared to the same period in 2018 (71.525 thousand).

In the last decades, it is noted that the diseases transmitted by the *Aedes aegypti* mosquito, specifically dengue, have grown exponentially worldwide. There are approximately 390 million dengue infections per year, including 96 million with clinical manifestations (Braht et al., 2013).

Among the methods used to control the larvae, the use of temephos organophosphate insecticide is the main measure adopted by the National Dengue Prevention Program in Brazil and by the World Health Organization (Carvalho et al., 2004; Crivelenti et al., 2011; Prophiro et al., 2011).

In view of the importance of EOs and their wide application, the present study aims to determine the chemical constituents and the larvicidal potential of the essential oil of *Plectranthus amboinicus* (Lour.) Spreng against the larvae of *Aedes aegypti*, aiming at a safe, ecologically viable and efficient alternative fighting and controlling the population of *Aedes aegypti* in the country.

2. Methodology

2.1 Obtaining plant material

Leaves of *Plectranthus amboinicus* (Lour.) Spreng (thick-leaf mint) in the city of Palmeirândia (MA), being transported to the Laboratory of Research and Application of Essential Oils of the Federal University of Maranhão (LOEPAV / UFMA), where they were dried, crushed and stored for later analysis.

2.2 Phytochemical screening

A hydroalcoholic extract of the plant material obtained was prepared and it was subjected to chemical tests based on the methodology presented by Matos (2009). The tests performed to identify alkaloids, steroids, phenolics, flavonoids, glycosides, cardiac glycosides, saponins and tannins are described below:

2.2.1 Steroids (Salkowsk test)

About 100 mg of dry extract was dissolved in 2 ml of chloroform. Sulfuric acid was carefully added to form a lower layer. A reddish-brown color at the interface indicated the presence of a steroid ring.

2.2.2 Alkaloids (Mayer's test)

1.36 mg of mercury chloride were dissolved in 60 ml and 5 mg of potassium iodide dissolved in 10 ml of distilled water, respectively. These two solvents were mixed and diluted to 100 ml using distilled water. To 1 ml of the aqueous acidic solution of the samples, a few drops of the reagent previously prepared were added. The formation of white or pale precipitation showed the presence of alkaloids.

2.2.3 Flavonoids

In a test tube containing 0.5 mL of alcoholic extract from the samples, 5 to 10 drops of diluted HCl were added and a small amount of Zn or Mg was added to the solution, which was then boiled for a few minutes. The appearance of a reddish

pink or dark brown color indicated the presence of flavonoids.

2.2.4 Glycosides

One small amount of alcoholic extract from samples was dissolved in 1 ml of water and then aqueous sodium hydroxide was added. The formation of a yellow color indicated the presence of glycosides.

2.2.5 Cardiac glycosides

About 100mg of extract was dissolved in 1ml of glacial acetic acid containing a drop of ferric chloride solution and 1ml of concentrated sulfuric acid was added. A brown ring obtained at the interface indicated the presence of an oxy sugar characteristic of cardenolides.

2.2.6 Saponins

A drop of sodium bicarbonate was added to a test tube containing about 50 ml of an aqueous extract from the sample. The mixture was shaken vigorously and held for 3 minutes. A honey comb like foam was formed and showed the presence of saponins.

2.2.7 Phenols

For 1 mL of alcoholic sample solution, 2 mL of distilled water was added followed by a few drops of 10% aqueous ferric chloride solution. The formation of a blue or green color indicated the presence of phenols.

2.2.8 Tannins

In a test tube containing about 5 ml of an aqueous extract, a few drops of 1% lead acetate solution were added. The formation of a yellow or red precipitate indicated the presence of tannins.

2.3 Obtaining essential oils

For the extraction of essential oils, the hydrodistillation technique was used with a Clevenger glass extractor coupled to a round bottom flask wrapped in an electric blanket as a heat generating source. 200g of *P. amboinicus* leaves were used, adding distilled water (1:10). Hydrodistillation was carried out at 100 ° C for 3 hours, collecting the extracted essential oil. Each essential oil was dried by percolation with anhydrous sodium sulfate (Na₂SO₄) and stored in a refrigerator until further analysis.

2.4 Chemical constituents

The EO constituents were identified by gas chromatography coupled to mass spectrometry (GC-MS). The conditions of analysis were as follows: Method: Adams. M; Injected volume: 0.3 µL; Column: Capillary HP-5MS (5% diphenyl, 95% dimethyl polysiloxane) (Equivalent DB-5MS or CP-Sil 8CB LB / MS), in dimensions (30 mx 0.25 mm x 0.25 µm); Drag gas: He (99,9995); 1.0 mL / min; Injector: 280 oC, Split mode (1:10); Oven: 40 oC (5.0 min.) Up to 240 oC at the rate of 4 oC .min⁻¹, from 240 oC to 300 oC (7.5 min) at the rate of 8 oC.min⁻¹; tT = 60.0 min; Detector: EM1; EI (70 eV); Scan mode (0.5 sec / scan); Mass range: 40 - 500 daltons (one); Line transfer: 280 oC .; Filament: off 0.0 to 4.0 min; Linear quadrupole mass spectrometer. The AMDIS (Automated Mass spectral Deconvolution Mass & Identification System) program was used to identify the compounds in the sample.

2.5 Egg collection

The eggs were collected at the Federal University of Maranhão, Campus Bacanga in São Luís / MA, using traps called ovitrampas. These consist of brown buckets (500 mL), made of polyethylene, with 1 mL of brewer's yeast and 300 mL of running water and two Eucatex straws are inserted for the mosquito's oviposition. The traps were inspected weekly for the replacement of straws and collection of eggs and sent to the Laboratory of Research and Application of Essential Oils (LOEPAV / UFMA) of the Technological Pavilion of the Federal University of Maranhão - UFMA.

Initially, the eggs of *Aedes aegypti* were placed to hatch at room temperature in a circular glass aquarium containing mineral water. The identification of the species followed the methodology proposed by Forattini (1962). The obtained larvae were fed with cat food according to the methodology of Silva et al., (1995) until they reached the third and fourth stages, the age at which the experiments were carried out.

2.6 Larvicidal activity

The tests for larvicidal activity were performed according to the adapted methodology proposed by Silva (2006). Initially, a stock solution of 100 mg L⁻¹ of each of the EOs was prepared and diluted in a 2% DMSO solution. From this solution, five dilutions were prepared at concentrations 10, 20, 50, 70 and 90 mg L⁻¹. At each concentration, 10 larvae were added in the proportion 1 ml per larva.

All tests were performed in triplicates and as a negative control, a solution formed of DMSO 2% was used, and as a positive control, a solution of temephos (O, O, O', O'-tetramethyl O, O'-tiodi-p -phenylene bis (phosphorothioate) at 100 ppm, equivalent to the concentration used by the National Health Foundation (FUNASA) for the larvicidal control of the vector, in addition to Novaluron (\pm -1- [3-chloro-4- (1-1-3 -trifluro-2-trifluoromethoxyethoxy) phenyl-3- (2,6-diflurobenzoyl) urea at 0.02 mg L⁻¹, dose adopted by the Ministry of Health, which indicates by the WHO in the range of 0.01 to 0.05 mg L⁻¹.

After 24h the counting of alive and dead was performed, being considered dead, the larvae that did not react to the touch after 24 hours of the beginning of the experiment. To quantify the efficiency of the EOs, the statistical test of Probit (Finney, 1952) was applied.

3. Results and Discussion

3.1 Phytochemical screening

Phytochemical analysis enabled the determination of secondary metabolites present in the analyzed plant materials, shown in Table 1.

Table 1. Phytochemical screening of *P.amboinicus*.

Species	Classes							
	1	2	3	4	5	6	7	8
<i>P. amboinicus</i>	+	-	+	+	+	+	+	+

Note: 1 - Steroids; 2 - Alkaloids; 3-Flavonoids; 4-Glycosides; 5-Cardiac glycosides; 6-Saponins; 7-Phenols; 8-Tannins. Source: Authors (2021).

Table 1 shows the presence of several classes in all analyzed plant materials. The presence of phytochemicals is largely influenced by several factors, including variety, genetic factors, maturation stage, climatic and edaphic

conditions. According to Menezes Filho and Castro (2019), temperature, water availability, ultraviolet radiation, addition of nutrients, environmental pollution and attack of pathogens are factors that can influence the amount of secondary metabolites present in plant extracts. According to Alfaia (2016), the natural function of many secondary metabolites has been investigated with greater tenacity, being recognized that these are essential for the existence of plants and in several biotechnological applications.

3.2 Chemical constituents

According to Table 2, the substances identified in GC / MS, it is possible to highlight that Carvacrol is the major component (69.93%), followed by Cyclohexanone (11.07%) and Caryophyllene (5.64%).

These results are in agreement with the study by (Monzote et al., 2020), who also found a high percentage in the aerial parts of *P. amboinicus* with a 71% content of carvacrol. The chemical composition of the essential oil of *P. amboinicus* is influenced by the extraction methodology and the seasonality of the plant (de Freitas Bezerra et al., 2017).

De Freitas Bezerra et al., (2017) observed that the extraction of mint leaves by hydrodistillation showed better results, when compared to steam distillation. The content of carvacrol reached 68% in the period of October 2014, being the major component and the Caryophyllene was quantified with 8.1% in the same period, presenting results similar to this study.

Table 2. Chemical constituents identified in the essential oil of *P. amboinicus*

ID	RT (min)	Content%	Compounds
1	15,016	0.98	Terpinen-4-ol
2	16,626	0.86	Tymol
3	16,788	69.93	Carvacrol
4	17,383	0.90	Piperitenone
5	17,676	11.07	Cyclohexanone
6	18,528	5.64	Caryophyllene
7	18,645	3.31	Bergamotene
8	18,999	1.94	α -Caryophyllene
9	19,318	0.94	Germacrene D
10	19,592	0.77	β -Bisabolene
11	20,595	3.66	Caryophyllene oxide

Source: Authors.

However, in a study by Santos et al., (2015), results inferior to this study were quantified in relation to the chemical composition of the leaves of *P. amboinicus*, with carvacrol being 37.7%, caryophyllene and cyclohexanone were not quantified. The same was presented by Huang et al., (2019), with a content of carvacrol of 61.53%, and the chemical components caryophyllene and cyclohexanone were also not found in this study. These results confirm the presence of a satisfactory percentage of the chemical components observed in the EO of this study.

3.3 Larvicidal activity in vitro front *Aedes aegypti*

Table 3 shows the LC₅₀ and LC₉₀ referring to the action of the EO against the larvae of *Aedes aegypti* calculated through the log of the intersection of the curves.

Table 3. Mortality *Aedes aegypti* for action of essential oil.

Log C	Mortality	LC ₅₀ mg L ⁻¹	LC ₉₀ mg L ⁻¹	χ ²	σ	R ²
1.00	19.0					
1.30	34.8					
1.48	47.5					
1.70	50.7	28.52	49.55	0.997	0.390	0.874
1.78	69.7	(21.39-38.03)	(40.31-62.33)			
1.85	88.7					
1.95	95.0					
2.00	95.0					

Source: Authors.

Regarding the larval activity of *P. amboinicus* EO, it is possible to observe that through Table 3, the plant presented LC₅₀ of 28.52 mg L⁻¹ and LC₉₀ of 49.55 mg L⁻¹ against the larvae of the mosquito *Aedes aegypti*.

According to the criteria of Dias and Moraes (2014), essential oils with lethal concentrations (LC₅₀) below 50 mg L⁻¹ are classified as highly active, to be considered active they must have LC₅₀ below 100 mg L⁻¹ and inactive when they have higher LC₅₀ at 100 mg L⁻¹, confirming the larvicidal potential of the EO assessed in this study.

These results are in agreement with the findings in the literature, which confirm the potential of *P. amboinicus* EO. Paramasivam et al., (2020) when assessing the larvicidal activity of extracts of *P. amboinicus*, collected in India, observed maximum activity with minimum concentrations against the larvae of the mosquito of the 4th instar of *Aedes aegypti*, with LC₅₀ ranging between 53, 36 and 13.64 µg mL⁻¹ and LC₉₀ of 92.51 and 86.09 µg mL⁻¹.

In a recent study by Huang et al., (2019), they obtained a higher value than this study, with an LC₅₀ of 42.90 mg L⁻¹, in the evaluation of the larvicidal action of the peppermint EO, against the larvae of the mosquito *Aedes aegypti*. The same was reported by dos Santos et al., (2020) who obtained an LC₅₀ of 41.7 mg L⁻¹ for the larvicidal activity of *P. amboinicus* EO, however, they are classified as highly active, as in this study.

In a study by Jayaraman, Senthilkumar and Venkatesalu (2015), they observed that after 12h, the larvicidal potential of the methanol extract of *P. amboinicus* against *Aedes aegypti* larvae was 322.67 ppm (LC₅₀) and 601.93 ppm (LC₉₀), showing a lower action when purchased at this time. study.

The use of *P. amboinicus* in traditional medicine, in other words, its biological action is related to the presence of some chemical components, such as carvacrol, thymol, phenols and aromatic acids, being very common its application as suddenly natural, as it has a high content of EO on its leaves (Huang et al., 2019).

4. Conclusion

These findings encourage the application of the EO of *P. amboinicus* in this study as larvicide, as it presents in greater quantity one of the chemical substances (carvacrol), which may be associated with its satisfactory biological action, thus allowing the development of an insecticide without effects collateral.

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