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Larvicidal activity opposite *Aedes aegypti* of the essential oil of the dry leaves of *Syzygium cumini* (L.) Skeels (Myrtaceae)

Atividade larvicida frente *Aedes aegypti* do óleo essencial das folhas secas de *Syzygium cumini* (L.) Skeels (Myrtaceae)

Actividad larvicida frente a *Aedes aegypti* del aceite esencial de las hojas secas de *Syzygium cumini* (L.) Skeels (Myrtaceae)

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Abstract

This study aimed to evaluate the larvicidal activity of essential oil (EO) extracted from the dry leaves *of Syzygium cumini* (L.) Skeels. The hydrodistillation technique was used for EO extraction in a modified Clevenger extractor system. The chemical constituents were obtained by Gas Chromatography coupled to mass spectrometry (GC/MS). For larvicidal activity, *Larvae of Aedes aegypti* were submitted to OE solutions at concentrations of 10-100 $mg^{L-1,}$where larvae mortality was evaluated and LC_{50} was determined. Isocaryofylene was quantified as the majority in THE. The action of the EO was observed as larvicidal where

$CL_{50\ of}$ 29.58 mg L^{-1} was determined. The results obtained allowed us to conclude that the studied OE presented efficient larvicidal activity against *the larvae of Aedes aegypti*, being important and encouraged its use and application.

Keywords: Larvicidal; Essential oil; Syzygium cumini.

Resumo

Este estudo objetivou avaliar a atividade larvicida do óleo essencial (OE) extraído das folhas secas de *Syzygium cumini* (L.) Skeels. Para extração do OE foi utilizada a técnica de hidrodestilação em um sistema extrator de Clevenger modificado. Os constituintes químicos foram obtidos por Cromatografia Gasosa acoplada a espectrometria de massas (CG/EM). Para atividade larvicida 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 CL_{50}. O isocariofileno foi quantificado como majoritário no OE. Observou-se ação do OE como larvicida onde foi determinada a CL_{50} de 29,58 mg L^{-1}. Os resultados obtidos permitiram concluir que o OE estudado apresentou atividade larvicida eficiente contra as larvas de *Aedes aegypti*, sendo importante e incentivado seu uso e aplicação.

Palavras-chave: Larvicida; Óleo essencial; Syzygium cumini.

Resumen

Este estudio tuvo como objetivo evaluar la actividad larvicida del aceite esencial (AE) extraído de las hojas secas de *Syzygium cumini* (L.) Skeels. La técnica de hidrodestáltica se utilizó para la extracción de AE en un sistema de extractor Clevenger modificado. Los componentes químicos se obtuvieron mediante cromatografía de gases acopladas a espectrometría de masas (GC/MS). Para la actividad larvicida, las larvas de *Aedes aegypti* se sometieron a soluciones de OE a concentraciones de 10-100 mg de L^{-1}, donde se evaluó la mortalidad de las larvas y se determinó LC_{50}. Isocaryofylene se cuantificó como la mayoría en the AE. La acción de la AE se observó como larvicida en la que se determinó la LC_{50} de 29,58 mg L^{-1}. Los resultados obtenidos nos permitieron concluir que el AE estudiado presentaba una actividad larvicida eficiente contra las larvas de *Aedes aegypti*, siendo importante y fomentaba su uso y aplicación.

Palabras clave: Larvicida; Aceite esencial; Syzygium cumini.

1. Introduction

The family Myrtaceae comprises about 121 genera with 3800 to 5800 species of shrubs and trees distributed mainly in tropical and subtropical areas of the world (Stefanello et al., 2011; Singh et al., 2015). The genus *Syzygium,* one of the main members of this family, covers 1,100 species with attention deserving *of Syzygium cumini* (L.) Skeels, a perennial tree commonly known as jambolão in Brazil (Faria et al., 2011) that has been used to treat numerous diseases (Ayyanar & Subash-Babu, 2012).

Phytochemical investigations reported that *the leaves of S. cumini* contain several terpenoids, alkaloids, lignanas and phenolics, including quercetin, myricetin, sitosterol, myricetin and betuline acid (Mir et al., 2009; Srivastava & Chandra, 2013). Extracts from different parts *of S. cumini,*such as fruits, seeds, leaves and stem bark, have been used for various pharmacological actions, as they are rich in active constituents with antimicrobial properties (Ayyanar et al., 2013). In addition, this species has been used in the treatment of leishmaniasis (Ayyanar & Subash-Babu, 2012) and chronic diarrhea (Veigas et al., 2007).

In addition, the leaves are rich in essential oil, with a prevalent presence of terpenes such as α-pineno, β-pineno, α-limoneno, α-cadinol, pinocarvon, pinocarveol (Mohamed et al., 2013). Dias et al. (2013) points out that the most abundant compounds in the essential oil of *S. cumini leaves* are monoterpenes (87.12%), with α- pineno (31.85%), (Z) - β -ocimene (28.98%) and (E)-β-ocimeno (11.71%). The authors also confirmed the leishmanicide biological action of the EO against promastigote forms at relatively low concentrations (Dias et al., 2013).

Note that despite all the benefits mentioned above for the EO of *S. cumini* has still been little used in the industry for the manufacture of products with beneficial health characteristics. Chhikara et al. (2018) encourages, from the observation of bioactive compounds and the effect on *the health of S. cumini,* this research seeks a study to increase its use in chemical and pharmaceutical products through biological potentials little studied for the species and restricted in the use of its essential oil.

Natural products obtained from medicinal plants provide a rich source of biologically active monoterpenes and are well documented for bioactivities against insects in all their phases (Govindarajan et al., 2012). Chemical constituents isolated from these products such as 1,8-cineol have a remarkable effect against larvae of *Aedes aegypti* (Araújo et al., 2003). Just like Chantraine et al. (1998) report that monoterpenes (*E*)-anetol and *E*-nerolidol were considered the most toxic active ingredients extracted from various bolivian plant

species. E-aetol was one of the most effective larvicide constituents used against *Aedes aegypti* (Cheng et al., 2004). Studies that prove the use of natural products in larvicidal control of transmitting species of various problematic diseases of world health.

A large number of studies related to the *genus Aedes* are observed, with emphasis *on Aedes aegypti* as a result of it being among the main public health problems worldwide. In recent decades, it has been found that diseases transmitted by the *Aedes aegypti*, especially dengue, have grown high worldwide. For 12 years, the World Health Organization (WHO) estimated that approximately 1.3 million individuals were at risk of being infected with the dengue virus (WHO, 2012). Currently, it is estimated that two-fifths of the world's population, that is, more than 2.5 billion people are subject to being infected with the dengue virus and who estimates that there may be about 50 million dengue infections per year worldwide (Zara et al., 2016). Thus, this study aimed to evaluate the chemical constitution and larvicidal potential of essential oil from *S. cumini* leaves in front of Larvae of *Aedes aegypti*.

2. Methodology

2.1 Botanical material

The collection of plant material used in this research was carried out in October 2019. The leaves of *S. cumini* L. at the Federal University of Maranhão. The plant materials were identified by the Herbarium Ático Seabra of the Federal University of Maranhão, under the register of n°1069 for *S. cumini*. After collection, the plant species were transported to the Laboratory of Research and Application of Essential Oils (LOEPAV/UFMA), where it was submitted to the convective air-drying oven FANEM 520 to 45ºC for 24 hours, and then crushed in a knife mill.

2.2 Essential oils

For the extraction of EOs, the hydrodistillation technique was used with a glass Clevenger extractor coupled to a round bottom balloon packed in an electric blanket as a heat generating source. 200g of the dried leaves of *S. cumini* were used, adding distilled water (1:10). Hydrodistillation was conducted at 100°C for 3h and the extracted EO was collected. Each EO was dried by percolation with anhydrous sodium sulfate (Na_2SO_4) and centrifuged. These operations were performed in triplicates and samples stored in amber glass ampoules under 4°C refrigeration. Subsequently submitted the analyses.

2.3 Chemical Analysis

The constituents of the EOs were identified by gas chromatography coupled to mass spectrometry (CG-MS) in the Fuel, Catalysis and Environmental Center (NCCA) of the Federal University of Maranhão (UFMA). 1.0 mg of the sample was dissolved in 1000 µL of dichloromethane (purity 99.9%). The conditions of analysis were as follows: Method: Adams. M, 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 m x 0.25 mm x 0.25 µm); Drag gas : He (99.9995); 1.0 $mL.min^{-1}$; Gun: 280 oC, Split mode (1:10); Oven: 40 oC (5.0 min.) up to 240 oC at a rate of 4 oC min^{-1}, from 240 oC to 300 oC (7.5 min) at a rate of 8 $oC.min^{-1}$); tT = 60.0 min; Detector : EM; EI (70 eV); Scan mode (0.5 sec $scan^{-1}$); 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.

2.4 Larvicidal Activity

The eggs were collected at the Federal University of Maranhão, Campus Bacanga in São Luís/MA, through traps called ovitrampas. These consist of brown buckets (500 mL), polyethylene, with 1 mL of beer yeast and 300 mL of running water and inserted two eucatex reeds for mosquito egg position. The traps were inspected weekly for the replacement of reeds and egg collection and sent to the Laboratory of Research and Application of Essential Oils (LOEPAV-UFMA) 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 larvae obtained were fed with cat feed according to Silva's methodology (1995) until they reached the third stage, the age at which the experiments were carried out.

The tests for larvicidal activity were carried out according to the adapted methodology proposed by Silva(2006). Initially, a 100 mg L^{-1} mother solution of EO diluted in 2% dimethylsulfoxide solution (DMSO) was prepared. Five dilutions were prepared from this solution at concentrations 10, 20, 50, 70 and 100 mg L^{-1}. At each concentration, 10 larvae were added in the proportion 1 mL/larva. All tests were performed in triplicates and as negative control was used a solution formed of DMSO 10%, and as a positive control, a solution of temephos (O,O,O',O'- tetramethyl O,O'-thiodi-p-phenylene bis (phosphothiotioate)

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 (±-1-[3-chloro-4-(1-1-3-trifluro-2-trifluoromethoxyethoxy) phenyl3-(2,6-diflurobenzoyl) urea at 0.02 mg/L, a dose adopted by the Ministry of Health, which indicates by the WHO in the range of 0.01 to 0.05mg/L.

After 24 hours, the live and dead were found, and larvae that did not react to the touch after 24 hours of the beginning of the experiment were considered dead. To quantify the efficiency of the EO, the Statistical Test of Reed & Muench(1938) was applied with calculation of the confidence interval by Pizzi(1950).

3 Results and Discussion

3.1 Chemical constituents

Table 1 presents the 28 chemical constituents identified in the EO of the leaves of *S. cumini*. 28 constituents were identified and the majority were: isocaryophyllene (18.01%), naphthalene (17.37%) and longifolene (11.65%).

Table 1: Chemical constituents identified in the EO of *S. cumini.*

RT (min)	Constituents	Class	%A
7,523	1- (1-methyl-2-cyclopenten-1-yl) -ethanone	Monoterpene	0,28
8,798	dimer β-pinene	Monoterpene	9,61
9,817	α-sabinene	Monoterpene	0,33
10,896	d-Limonene	Monoterpene	0,29
15,910	p-ment-3-ene	Monoterpene	1,50
16,079	α-cubebeno	Sesquisterpene	1,50
16,411	α-copaene	Sesquisterpene	1,07
16,500	β-copaene	Sesquisterpene	3,21
16,665	guaia-10 (14), 11-diene	Sesquisterpene	3,44
16,913	4-aromadendrene	Sesquisterpene	0,70
17,120	isocaryofylene	Sesquisterpene	18,01

17,240	sesquiterpene	Sesquisterpene	1,68
17,285	α-guaieno	Sesquisterpene	0,49
17,365	spathulenol	Sesquisterpene	1,40
17,489	isogermacreno D	Sesquisterpene	0,22
17,591	α-humulene	Sesquisterpene	2,62
17,805	γ-cadinene	Sesquisterpene	8,28
17,860	naphthalene (isomer)	Monoterpene	1,44
17,913	naphthalene (isomer)	Monoterpene	17,37
18,029	virdifloreno	Sesquisterpene	3,79
18,080	longifoleno (V4)	Sesquisterpene	11,65
18,298	(+) - δ-cadinene	Sesquisterpene	2,79
18,339	(+) - δ-cadinene (isomer)	Sesquisterpene	2,41
18,394	calameneno	Sesquisterpene	0,59
18,810	nerolidol	Sesquisterpene	0,54
18,901	γ-elemene	Sesquisterpene	1,78
19,110	diethyl phthalate	Monoterpene	2,35
19,190	caryophyllene oxide	Sesquisterpene	0,66

Source: Author, (2020).

A significant variation in the chemical composition of the essential oil extracted from the leaves of *Syzygium cumini* was also observed. Shafi et al. (2002) reported that the essential oil of jamelon leaves collected in Southern India was abundant in pinocarveol (15.1%), α-terpineol (8.9%), myrtenol (8.3%), eucarvone (6.6%) and murolol (6.4%). Nishandhini et al. (2015) describe for an oil sample obtained from *specimens of Syzygium cumini* from the same country, an abundance in α-pineno (21.5%), b-ocimeno (E) (6.8%), α-terpineol (9.5%) and δ-cadineno (8.3%).

In another study Corrêa et al. (2018) revealed with the analysis of chromatogram and spectra obtained, the identification of some components in volatile oil, such as: a-pineno, a-tujona, ß-pineno, ß-myrcene, p-cymene and other terpenic compounds.

In Xavier (2019) chromatographic analysis of *S. cumini* essential oil, 56.25% monoterpenes and 43.75% sesquiterpenes were expressed, corroborating Ruggiero

(2004), which obtained a higher proportion of monoterpenes and diverging from Ucker (2016) who reported higher amounts of sesquiterpenes (55.55%). The studies of Dias et al. (2013), Silva et al. (2018) and Saroj et al. (2015) point out that these analyses present some differences in the percentage of compounds and/or chemical composition of essential oil, which is justified by Debbarma et al. (2012), which describes that the chemical profile depends on the genetic nature of the plant, harvest time, geographical conditions, luminosity, among others.

3.2 Larvicidal activity

The larvicidal activity of *the essential oil of S. cumini* with the data obtained regarding the number of live larvae and dead larvae were found by an average of the three replicates for each of the six concentrations tested, are presented in Table 2.

Table 2: Lethal Concentration 50% and 90% for EO action against *Aedes aegypti*

Concentration mg L^{-1}	**%Mortality**	**LC_{50}**	**LC_{90}**	**Classification**
100	100	29,58 ± 1,71 mg L^{-1}	50,91 ± 1,71 mg L^{-1}	Efficient (potential for encouraged application) Cheng et al. (2003)
85	85			
70	75			
50	55			
30	45			
10	25			

Source: Author, (2020).

The concentration of 10 mg L^{-1} of the EO of *H. courbaril* L. showed a low larvicidal activity, corresponding to 25% mortality. The concentration of 30 mg L^{-1} presented a mortality of 45%. From the concentration of 50 mg L^{-1} of EO larvicidal activity began to grow exponentially, until reaching the concentration of 100 mg L^{-1}, where it caused the death of 100% of the larvae. The LC_{50} for the EO of *S. cumini* was calculated by the intersection of the curves of living accumulated individuals and accumulated individuals, resulting in LC_{50} of 29.58 mg L^{-1} with a confidence interval of 1.71 mg L^{-1} presented in Table 2.

The LC_{50} observed in this study for *S. cumini* encourages the use of essential oil, since a LC_{50} of 223.9 mg/L and LC_{90} of 524.8 mg/L was observed in the study by Kaushik&Saini(2009), much higher results for the authors who used the plant extract of the species. However, in the study by Murthy&Rani(2009) a LC_{50} lower than 100 mg/L was observed, being classified as well as in this study. The study of natural products obtained from plants with larvicidal *activity against Aedes aegypti* is recent, and studies carried out in order to isolate and characterize such bioactive substances from the 1980s on. Most studies are conducted with crude extracts and essential oils, and in most of these cases, the compound responsible for the activity presented is not known (Braz-Filho, 1994; Schenkel et al., 2003).

3. Final Considerations

The essential oil of *S. cumini* showed efficient larvicidal activity against *the larvae of Aedes aegypti*. where the criterion used considers good larvicide agents' substances with a value of CL_{50} below 100 mg L^{-1}. Thus, evidencing its potentiality and being an alternative in the control of *larvae of Aedes aegypti*.

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Francilidia Oliveira Vitorino de Assunção Conceição – 4%

Liane Batista da Cruz Soares – 4%

Maria Gizelda Gomes Lages – 4%

Feliciana Santos Pinheiro – 4%

Ricardo Teixeira de Sousa – 4%

Ione Rocha Neves – 4%

Thayson Rodrigues Lopes –4%

Victor Elias Mouchrek Filho – 20%