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Essential oils of the leaves of *Syzygium cumini* (L.) Skeels and fruit peels of *Hymenaea courbaril* (L.) var. courbaril as molluscides against *Biomphalaria glabrata*

Óleos essenciais das folhas de *Syzygium cumini* (L.) Skeels e cascas do fruto de *Hymenaea courbaril* (L.) var. courbaril como moluscicidas contra *Biomphalaria glabrata*

Aceites esenciales de hojas de *Syzygium cumini* (L.) Skeels e *Hymenaea courbaril* (L.) var peels. courbaril como molusquicidas contra *Biomphalaria glabrata*

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Abstract

This study evaluated the molluscicidal activity of essential oils (EOs) of *Syzygium cumini* L. and *Hymenaea courbaril* L. Hydrodistillation was used to extract the EOs. The chemical characterization was performed by Gas Chromatography coupled to mass spectrometry. The bioassay of *Artemia salina* Leach was used for the toxicity test. The molluscicidal assay tested concentrations of 10-80 mg L^{-1} of the EOs against *Biomphalaria glabrata*. The major constituent of The EO of *S. cumini* was isokaryophyllene and *H. courbaril* the Germacreno-D. The toxicity assay classified The EOs as nontoxic with LC_{50} 412.10 mg L^{-1} for *S. cumini* and

LC_{50} 354.80 mg L^{-1} for *H. courbaril*. The molluscicidal assay purchased LC_{50} 44.76/ LC_{90} 77.20 for *S. cumini* and LC_{50} 37.34 mg L^{-1}/ LC_{90} 73.24 mg L^{-1} for *H. courbaril*. The results showed that both EOs can be used as natural molluscicides in combating the snail *Biomphalaria glabrata*.

Keywords: Essential oils; *Syzygium*; *Hymenaea.*

Resumo

Este estudo avaliou a atividade moluscicida de óleos essenciais (OE's) de *Syzygium cumini* L. e *Hymenaea courbaril* L. A hidrodestilação foi usada para extrair os OE's. A caracterização química foi realizada pela Cromatografia Gasosa acoplado à espectrometria de massa. O bioensaio de *Artemia salina* Leach foi utilizado para o teste de toxicidade. O ensaio moluscicida testou concentrações de 10-80 mg L-1 dos OE's contra *Biomphalaria glabrata*. O principal constituinte do OE de *S. cumini* foi isocariofileno e *H. courbaril* o germacreno-D. O ensaio de toxicidade classificou os OE's como não tóxicos com LC_{50} 412,10 mg L^{-1} para *S. cumini* e LC_{50} 354,80 mg L^{-1} para *H. courbaril*. O ensaio moluscicida comprovou LC_{50} 44.76/ LC_{90} 77,20 mg L^{-1} para *S. cumini* e LC_{50} 37.34/ LC_{90} 73,24 mg L^{-1} para *H. courbaril*. Os resultados mostraram que ambos os OE's podem ser usados como moluscicidas naturais no combate contra o caracol *Biomphalaria glabrata*.

Palavras-chave: Óleo essencial; *Syzygium*; *Hymenaea.*

Resumen

Este estudio evaluó la actividad moluscicida de los aceites esenciales (AE's) de *Syzygium cumini* e *Hymenaea courbaril*. La hidrodestestación se utilizó para extraer los AE's. La caracterización química fue realizada por Cromatografía de Gases acoplada a Espectrometría de Masas. El bioensayo de *Artemia salina* Leach se utilizó para la prueba de toxicidad. El ensayo con moluscicidal aprobó concentraciones de 10-80 mg de L^{-1} de las AE's contra *Biomphalaria glabrata*. El componente principal de la AE de *S. cumini* fue isokaryophyllene y *H. courbaril* el Germacreno-D. El ensayo de toxicidad clasificó a los AE's como no tóxicos con LC_{50} 412.10 mg L^{-1} para *S. cumini* y LC_{50} 354.80 mg L^{-1} para *H. courbaril*. El ensayo con moluscicidal compró LC_{50} 44,76/ LC_{90} 77,20 mg L^{-1} para *S. cumini* y LC_{50} 37,34/ LC_{90} 73,24 mg L^{-1} para *H. courbaril*. Los resultados mostraron que ambas AE's pueden utilizarse como molusquicidas naturales en la lucha contra el caracol *Biomphalaria glabrata*.

Palabras clave*:* Aceite esencial; *Syzygium*; *Hymenaea.*

1. Introduction

Schistosomiasis, popularly known as schist, snail disease or water belly, is a parasitic disease caused by helminth *Schistosoma mansoni* that has as its definitive host man and intermediate the mollusc of the *Biomphalaria glabrata* (Rocha et al., 2013). Historically, the nature of parasite-host relationships has attracted much attention from the scientific community, especially parasites that affect human populations, given the medical interest in controlling their transmission (Gashaw et al., 2015).

The transmission of parasitosis occurs through the human count with contaminated water in places where there are molluscs of the genus *Biomphalaria* spp. infected with the verme fences (Alves, 2018). *Biomphalaria* spp., are pulmonary and hermaphrodite organisms, which inhabit freshwater collections. Known for more than 140 million years, these molluscs have survived various environmental pressures and currently occupy large territorial tracts inhabiting preferably lentic water regions. In general, they are found in several collections such as rivers, ponds, ditches, streams, reservoir banks or artificial collections (Baron et al., 2013; Saúde, 2014; Standley, Goodacre, Wade, & Stothard, 2014).

According to WHO estimates, schistosomiasis is established in 76 countries, with an estimated 779 million people being at risk of infection and 207 million infected worldwide (Massara et al., 2012). In Brazil, this disease has already been described in 18 states and the Federal District, data indicate that between 2.5 and 6 million people are infected with the disease (Agudo-Padrón & Saalfeld, 2013; A. S. Dias, 2013; Saúde, 2014). The control of schistosomiasis requires measures that include treatment of carriers, basic sanitation, sanitary education and application of molluscicides.

The presence of schistosomiasis is an indicator of precarious conditions and this approach should be present in the minds of all specialists in the control of this endemic disease. Currently only the molluscicide niclosamide (Bayluscid®) has been used to combat disease vector snails (Rocha, Freitas, Azevedo, Souza, & Santos, 2014). However, the use of molluscicide of synthetic origin has generated concern regarding factors such as: toxicity to other species, low selectivity; contamination of the environment and the high cost of being imported (Cantanhede, Marques, Silva-Souza, & Valverde, 2010). In this context, the search for easily biodegradable substances has increased the interest in researchers in the search for natural products that present molluscicide activity. Compounds of plant origin with molluscicide potential have been widely used in the literature (Saúde, 2014). The World

Health Organization emphasizes the need for molluscicide studies of plants in order to make the control of schistosomiasis less costly and more efficient.

Brazil is the country with the largest diversity of plants in the world, with approximately 55,000 plant species cataloged from an estimated total of between 350,000 and 550,000 species worldwide (Teles, Ferreira, & Carvalho, 2014). The properties of these medicinal plants are directly related to their essential oils (EOs), which are a complex mixture of various active substances, including terpenes, which are formed by isoprelene units and phenylpropane derivatives (Ascenção & Mouchrek Filho, 2013).

Among the species rich in these aromatic compounds are the *Syzygium cumini* L. of the family of Myrtaceae popularly known as jambolan (Silva et al., 2012)and the species *Hymenaea courbaril* L. popularly known as "Árvore de Copal', Jatobá or Jutaí, belonging to the Fabaceae family (Fernandes et al., 2015). These species are characterized by having large amounts of phytochemicals, especially phenolic compounds and vitamin C (Oliveira et al, 2014). Despite their bioactive potential, few studies are conducted on these plants (Ramos et al., 2006). Thus, this study aimed to perform the chemical characterization, toxicity and evaluation of molluscicidal activity of essential oil extracted from the leaves of *S. cumini* and the bark of *H. courbaril* L.

2. Methodology

2.1. Botanical material

The collection of plant material used in this research was carried out in October 2019. The fruit peels of *H. courbaril* L. (jatoba) were collected in the region of Palmeirândia - MA and the leaves of *S. cumini* L. (jambolan) 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°1079 for *H. courbaril* 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* and 200g of the fruit peels *H. courbaril* 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. Toxicity

This test was performed according to the methodology described by Meyer et al. (1982). In a rectangular container, with a partition containing holes of approximately 0.02 cm thickness spaced by 0.5 cm and evenly distributed, artificial saline solution (60 g L^{-1} of distilled water) were added (60 g of sea salt/ 1L of distilled water). The container was placed inside an incubator illuminated by a fluorescent lamp, with aeration. On one side of this

container, about 64 mg of *Artemia salina* cysts were added, taking care that they did not cross the partition. The part of the system containing artemia saline cysts was covered with aluminum foil, so that the organisms, at birth, were attracted by light on the other side of the system, forcing them to cross the partition. This procedure aims at homogenizing the physical conditions of the test organisms. Incubation was performed for a period of 48h. Throughout the test the temperature was monitored.

For the evaluation of the lethality of *Artemia salina* Leach, a stock saline solution of each EO was prepared at the concentration of 10,000 mg L^{-1} and 0.02 mg of Tween 80 (active tense). Aliquots of 5, 50 and 500 µL of this were transferred to test tubes and completed with saline solution previously prepared up to 5 mL, obtaining concentrations of 10, 100 and 1000 mg L^{-1}, respectively. All tests were performed in triplicates, where ten larvae in the nauplium phase were transferred to each of the test tubes.

For white control, 5 mL of saline solution was used for positive control $K_2Cr_2O_7$ and for negative control 5 mL of a solution 4 mg L^{-1} of Tween 80. After 24 hours of exposure, the live larvae were counted, considering those that did not move during observation or with the slight agitation of the bottle. The criterion established by Dolabela(1997) was adopted to classify the toxicity of The EOs, being considered highly toxic when $LC_{50} \leq 80$ mg L^{-1}, moderately t vbboxic to 80 mg $L^{-1} \leq LC_{50} \geq 250$ mg L^{-1} and mildly toxic or nontoxic when $LC_{50} \geq 250$ mg L^{-1}.

The statistical analysis of the data for the LC_{50} was performed according to the method Reed&Muench (1938), from the table containing the mortality data for each concentration tested, a graph is constructed where a curve is observed for the accumulation of dead animals in each concentration log and another curve for the accumulation of survivors. The point of intercession between the curves is the Lethal Concentration 50% (LC_{50}), because at this point the number of surviving animals is equal to the number of dead animals. The confidence interval was calculated according to method Pizzi (1950) in which a graph of the percentage of dead versus log of the concentration is constructed.

2.5. Total phenolics

The determination of the total phenolic compounds of the EOs was performed with adaptation of the Folin-Ciocalteu method(Waterhouse, 2002). 5 mg of the essential oil diluted in 1 mL of ethanol was used. To this solution was added 3 mL of distilled water, 500 µL of folin-ciocalteu reagent and 2.0 mL of sodium carbonate at 20%. The solution formed was

taken to the water bath at 50 ºC for 5 min, removed and left to cool; and then, the reading was performed in a manual spectrophotometer, in a length of 760 nm. The standard curve was expressed in mg L^{-1} of tannic acid.

2.6. Antioxidant activity

The determination of antioxidant activity was performed by the ABTS method [2,2-azinobis-(3- ethylbenzothiazolin-6-sulfonic)], according to the methodology suggested by Re et al. (1999). The ABTS•+ radical was prepared by the 5.0 mL reaction of a 3840 µg mL^{-1} solution of ABTS with 88 µL of the 37,840 µg mL^{-1} potassium persulfate solution, the mixture was left in a dark environment for 16 hours. After radical formation, the mixture was diluted in ethanol until absorbance of 0.7±0.01 to 734 nm was obtained.

From the concentrations of The EOs (5 to 150 µg mL^{-1}) the reaction mixture with the radical cation ABTS was prepared. In a dark environment, an aliquot of 30 µL of each concentration of The EO was transferred in test tubes containing 3.0 mL of the radical Cation ABTS and homogenized in a tube agitator and after 6 minutes the absorbance of the reaction mixture was performed in spectrophotometer in length of 734 nm.

The capture of the free radical was expressed as a percentage of inhibition (%I) of the radical cation ABTS according to Equation 1 (Babili et al., 2011), where ABS_{ABTS} represents the absorbance of the ABTS radical solution and ABS_{AM} represents the absorbance of the sample.

$$\%InhibitionABTS = \frac{ABS_{ABTS} - ABS_{AM}}{ABS_{ABTS}} * 100 \qquad \text{Eq. 01}$$

From the obtained data, the efficient concentrations IC_{50} and IC_{90} were calculated, defined as the concentration of the sample necessary to kidnap 50% of the ABTS radicals. The EO is considered active when it presents $IC_{50}\% < 500$ µg mL^{-1}(Campos et al., 2003).

2.7. Obtaining and cultivating snails

Samples of snails of the species *Biomphalaria glabrata* were captured in rainy periods, in areas with low sanitation in the neighborhood Sá Viana, São Luís-MA. The collection technique was performed according to a proposal from Brazil(Epidemiológica, 2008) performing a scan with a shell in the submerged areas and the captured snails were

collected in a glass container with lid, with water from the breeding site itself. Their search was carried out at various points in each breeding site, and then sent to the molluscum of the Laboratory of Research and Application of Essential Oils (LOEPAV/UFMA).

The snails were kept in the laboratory for 30 days and analyzed every 07 days to confirm the absence of Infection by *Schistossoma mansoni*. For this, 05 snails were placed in transparent glass containers with 25 mL of dechlorinated water, that is, 5 mL/snail, exposed to light (60 W lamps) for one hour with a distance of 30 cm to stimulate the release of the fences and taken to be analyzed, through visualization with the aid of a stereoscopic magnifying glass (8x), those that were parasitized (positive) were labeled and separated for future individual analysis and those who showed no signs of trematoid infection in the period of 30 days were selected for the molluscicidal activity test.

2.8. Evaluation of molluscicide activity

For the evaluation of molluscicide activity, the technique recommended by the World Health Organization(1983) was used, where two tests were performed in triplicate. In the first, called a pilot test, a solution of the oil under study was prepared in a volume of 500 mL at a concentration of 100 mg. L^{-1} and 0.15 mL of Tween 80 (active tense), where 10 adult snails were placed, negative for Schistossoma mansoni, obtaining at the end a ratio of 50 mL/snail and feeding them with hydroponic lettuce.

They were exposed in the solution for 24 h, at room temperature, removed from the solution, washed twice with dechlorinated water, placed in a glass container containing 500 mL of dechlorinated water, feeding them with hydroponic lettuce and observed to every 24 hours for 4 days to assess mortality.

In the second test, called lethal concentration (LC_{50}), solutions of each oil were prepared in a volume of 500 mL at concentrations 100, 75, 62.5, 50, 20, 10, 5 and 2 mg L^{-1} and 0.15 mL of Tween 80 (surfactant), using the same methodology of the pilot test. For the negative control, two tests were also used, in the first we placed 500 mL of dechlorinated water and 10 snails in a glass container and in the second 10 snails immersed in a solution with 0.15 mL of Tween 80 in 500 mL of distilled water, feeding both with hydroponic lettuce and the analysis also performed in the previous tests.

The lethal concentration LC_{90} of the bioassay was determined by linear regression, obtaining the concentration versus mortality ratio of molluscs(Colegate&Molyneux, 2007). Mortality rates were obtained by averaging dead individuals as a function of the logarithm of

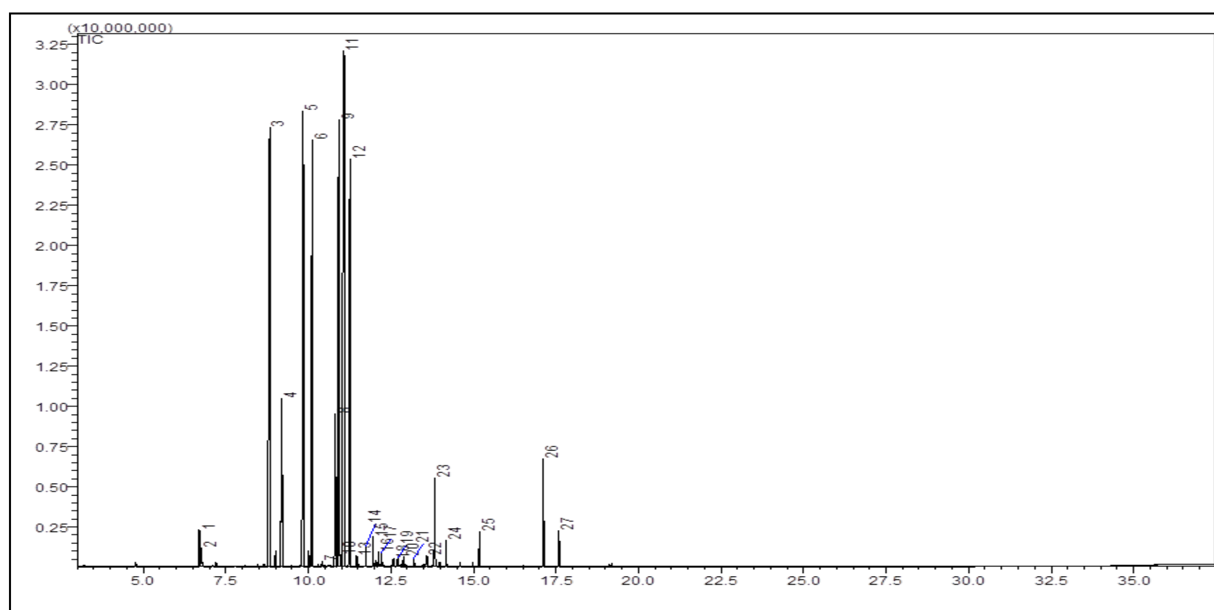
the tested dose. The statistical analysis of the data for the LC_{50} was performed according to the Reed&Muench method (Reed&Muench, 1938). The confidence interval was calculated according to method Pizzi (1950) in which a graph of the percentage of dead versus log of the concentration is constructed.

3. Results and Discussion

3.1. Chemical constituents

Figure 1 shows the EO chromatogram of *H. courbaril*.

Figure 1. *H. courbaril* EO chromatogram.



Source: Authors (2020).

Table 1 presents the 25 chemical constituents identified in the EO of *H. courbaril*.

Table 1. Chemical constituents identified in the EO of *H. courbaril.*

Order	RT (min)	Constituents NIST08	Class	%T
1	6,697	(E) -2-hexenal	Monoterpene	0,90
2	6,760	(Z) -hex-3-en-1-ol	Monoterpene	0,44
3	8,823	α-pinene	Monoterpene	12,33
4	9,196	canfeno	Monoterpene	4,31
5	9,843	β-pinene	Monoterpene	11,79
6	10,118	β-myrceno	Monoterpene	11,38
7	10,427	(E) -3-hexen-1-ol	Monoterpene	0,08
8	10,816	o-cymene	Monoterpene	3,36
9	10,925	d-Limonene	Monoterpene	13,51
10	10,976	eucalyptol	Monoterpene	0,20
11	11,085	β-ocimene	Monoterpene	23,33
12	11,261	β-cis-ocimene	Monoterpene	10,59
13	11,466	γ-terpinene	Monoterpene	0,20
14	11,745	α-pinene epoxide	Monoterpene	0,36
15	11,958	δ-carene	Monoterpene	0,56
16	12,122	α-pinene oxide	Monoterpene	0,26
17	12,207	linalool	Monoterpene	0,25
18	12,576	2-fencanol	Monoterpene	0,14
19	12,706	1-nonene-3-yne	Monoterpene	0,20
20	12,875	oxirane	Monoterpene	0,17
21	13,201	canfenilanol	Monoterpene	0,15
22	13,596	p-ment-1-en-4-ol	Monoterpene	0,16
23	13,826	α-terpineol	Monoterpene	1,56
24	14,173	phenyl acetate	Monoterpene	0,48
25	15,179	borneol	Monoterpene	0,63

Source: Authors (2020).

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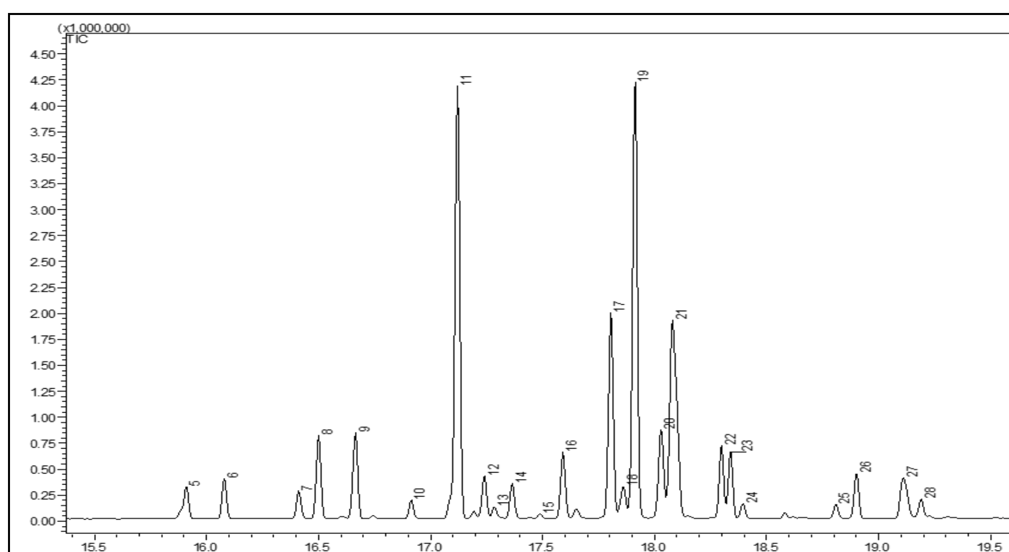
Sales et al. (2015) analyzed the chemical composition of *H. courbaril* EO from the dried and crushed peels of ripe fruits and found 28 chemical constituents, the majority of which were Germacrene-D (17.61%), (Z)-β-cayophyllene (17.56%), caryophyllene oxide (14.65%) and α-copaeno (8.46%). Mercês (2015) evaluated the chemical composition of EO of *H. courbaril* fruits, finding the following major chemical components: β-caryophyllene (43.7%) and cariophyllene oxide (11.9%). The biological properties of *H. courbaril* EO may be associated with the high concentration of hydrocarbon monoterpenes, especially β-cariophyllene. Mercês et al. (2018), we verified the chromatographic analyses performed in CG/MS of the EO of *H. courbaril* leaves, it was possible to detect 36 compounds, 26 were identified, representing 76.03% of the oil constituents. The analysis revealed the cariophyllene oxide and β-caylophyllene as major compounds of the EO of this species 20.63% and 16.78%, respectively.

According to Pereira et al. (2007), the composition of the EO of a species of this plant is related to environmental aspects, such as: the vegetative cycle of the plant; the process of obtaining essential oil; the environment in which the species develops, the type of cultivation, temperature, relative humidity, the amount of water and nutrients in the terrain, among others. This dependence on the composition of essential oil from environmental conditions can be verified by comparing the composition and yield of EOs extracted in different locations and parts of the plant.

Figure 2 shows the EO chromatogram of the leaves of *S. cumini*.

Figure 2. *S. cumini* EO Chromatogram.



Source: Authors (2020).

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Table 2 presents the 28 chemical constituents identified in the EO of the leaves of *S. cumini*.

Table 2. Chemical constituents identified in the EO of *S. cumini.*

Order	RT (min)	Constituents NIST08	Class	%T
1	7,523	1- (1-methyl-2-cyclopenten-1-yl) - ethanone	Monoterpene	0,28
2	8,798	dimer β-pinene	Monoterpene	9,61
3	9,817	α-sabinene	Monoterpene	0,33
4	10,896	d-Limonene	Monoterpene	0,29
5	15,910	p-ment-3-ene	Monoterpene	1,50
6	16,079	α-cubebeno	Sesquisterpene	1,50
7	16,411	α-copaene	Sesquisterpene	1,07
8	16,500	β-copaene	Sesquisterpene	3,21
9	16,665	guaia-10 (14), 11-diene	Sesquisterpene	3,44
10	16,913	4-aromadendrene	Sesquisterpene	0,70
11	17,120	isocariofileno	Sesquisterpene	18,01
12	17,240	sesquiterpene	Sesquisterpene	1,68
13	17,285	α-guaieno	Sesquisterpene	0,49
14	17,365	spathulenol	Sesquisterpene	1,40
15	17,489	isogermacreno D	Sesquisterpene	0,22
16	17,591	α-humulene	Sesquisterpene	2,62
17	17,805	γ-cadinene	Sesquisterpene	8,28
18	17,860	naphthalene (isomer)	Monoterpene	1,44
19	17,913	naphthalene (isomer)	Monoterpene	17,37
20	18,029	virdifloreno	Sesquisterpene	3,79
21	18,080	longifoleno (V4)	Sesquisterpene	11,65
22	18,298	(+) - δ-cadinene	Sesquisterpene	2,79
23	18,339	(+) - δ-cadinene (isomer)	Sesquisterpene	2,41

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24	18,394	calameneno	Sesquisterpene	0,59
25	18,810	nerolidol	Sesquisterpene	0,54
26	18,901	γ-elemene	Sesquisterpene	1,78
27	19,110	diethyl phthalate	Monoterpene	2,35
28	19,190	caryophyllene oxide	Sesquisterpene	0,66

Source: Authors (2020).

According to Table 2, the chemical composition of the EO of *S. cumini* leaves showed that the major components present were isokaryophyllene (18.01%), followed by naphthalene (17.37%), longifolene (11.65%), β-pinene dimer (9.61%) and other compounds at lower concentrations. Almeida(2019) identified 32 constituents, which reached 95.71% of the total leaf composition of *S. cumini*, of which α-pinene(10, 72%), (Z)-β-ocimeno (27, 98%), (E)-β-ocimeno (15, 48%) and β-Cariophyllene (13.79%) were the majority components. Dias et al. (2013) found 11 chemical compounds present in the EO of *S. cumini* leaves, reaching 99.98% of the total constitution, of which α-pinene (31.85%), (Z)-β-ocimeno (28.98%) and (E)-β-ocimen (11.71%) were the majority components found. According to Ramos et al. (2006) the presence of α-pinene and ocimene isomers are main constituents of EOs isolated from leaves, stems and fruits of *S. cumini*. Ucker(2016) evaluated the chemical composition of EO of jambolão seeds, 5 chemical components were found in the EO of jambolão seeds, it can be observed that the major compounds are β-cayophyllene (51.82%), followed by α-cayophyllene(27.05%) , β-pinene (7.76%) and α-pinene (6.56%). Evidencing the results of this study where β-caryophyllene presented itself as the major component.

The variability in the composition of the oil may be due to genetic factors, physiological and environmental factors. In fact, the chemical composition of EOs can be influenced by several factors, including harvest period, climate and geographical conditions, time of day of collection, plant distillation method, existence of chemotypes and vegetative stage of the plant(Sözmen et al., 2012).

3.2. Total phenolics of EOs

Table 3 shows the amount of total phenolics in the EOs of *H. courbaril* L and *S. cumini*. According to Table 3, the EO of *H. courbaril* presented 490,353 mg EAT g^{-1} in total phenolics. Veggi(2014) observed the total phenolic content of *H. courbaril* bark extract using

the Folin-Denis method, obtaining 335.0 mg EAT g^{-1}. Veggi et al. (2014) by analyzing the content of maximum total phenolic compounds in the extracts of the barks of *H. courbaril*, the quantification was observed in 335.00 mg of TAE / g of extract. Vecanto et al. (2016) found in the aqueous extract of the bark the value of 516.89 EAG g^{-1}.

Table 3. Total phenolics, mg EAT g^{-1}, in Eos.

EO	Total Phenolic Content
H. courbaril	490,353 mg EAT g^{-1}
S. cumini	578,453 mg EAT g^{-1}

Source: Authors (2020).

For the EO of *S. cumini*, the total phenolics were 578,453 mg EAT g^{-1}, according to Table 3. Thus, this study obtained superior results in relation to the analyzed one. Kaneria&Chanda (2013) quantificated the total phenolics of the extract obtained from a solvent sequence of the leaves of *S. cumini* in 104.42 (mg / g of extracted compound).

As can be seen, the EOs presented considerable concentrations of phenolic substances. The variation found in the studies can be explained by several factors, among them, the parts of the plant used, different polarities of the solvents used in the studies, as well as their environmental conditions, which results in the modification of the plant constituents present in the extracts (Gobbo-Neto & Lopes, 2007). Phenolic antioxidants are products of secondary metabolism of plants, and antioxidant activity is mainly due to their redox properties and chemical structure (Decker, 1997). EOs, as natural sources of phenolic components, attract researchers to evaluate their activity as antioxidants or free radical hijackers (Elansary et al., 2012). Phenolic compounds are also effective donors of hydrogen, which makes them good antioxidants (Rice-evans et al., 1995).

3.3. Antioxidant activity

The chemical complexity of EOs, usually a mixture of dozens of compounds with different functional groups, polarity and antioxidant activity that can lead to scattered results, depending on the test employed (Sacchetti et al., 2005), that's why we prefer to use ABTS and DPPH, widely used. Currently, there is no official method for determining antioxidant activity

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of plant origin and its derivatives due to the variety of antioxidant mechanisms that may occur and the diversity of bioactive compounds. The literature reports several methods for evaluating antioxidants, each with a different principle using free radicals and/or different patterns. Thus, studies aimed at evaluating the antioxidant properties of plant extracts need to use more than one method to accurately conclude that the extracts analyzed may also be able to combat the harmful effects of free radicals on the human body (Sousa et al., 2011).

Table 4. Antioxidant capacity of Eos.

EO	ABTS IC_{50}	%Inibição máxima	DPPH IC_{50}	%Inibição máxima
H. courbaril	10,12 µg mL^{-1}	99,10%	30,82 µg mL^{-1}	96,11%
S. cumini	7,05 µg mL^{-1}	98,45%	50,30 µg mL^{-1}	95,12%

Source: Authors (2020).

Table 4 shows that The EO of *H. courbaril* presents better antioxidant activity than The EO of *S. cumini*, but they are close in the values presented.

Vecanto et al. (2016) when analyzing the antioxidant potential of the aqueous extracts of the barks of *H. courbaril*, he observed activity with IC_{50}= 33.97 ± 0.55 µg mL^{-1}. Veras et al. (2020) analyzed the EO of *Hymenaea cangaceira* and observed its results for antioxidant activity with IC_{50} ranging from 127.51 ± 0.01 µg mL^{-1} to 467.29 ± 0.12 µg mL^{-1}. Farias et al. (2013) evaluated *H. courbaril* seed extract by finding IC_{50} 247.95 µg mL^{-1}.

The results presented for the antioxidant activity of *S. cumini* EO are satisfactory when compared to the Banerjee et al. (2005) study which evaluated the antioxidant activity of *S. cumini* fruit pulps and observed IC_{50} ranging in 168-428 µg mL^{-1}. Ruan et al. (2008) found that the methanol extract of the leaves of *S. cumini* has IC_{50} of 125.39 µg mL^{-1}. The antioxidant activity found by Elansary et al. (2012) of the EO of *S. cumini* leaves was attributed to the presence of a high percentage of α-terpineol (16.67%) and α-pinene (17.53%) .Eshwarappa et al. (2014) by analyzing the antioxidant activity of extracts from the branches of *S. cumini* and obtained the values of IC_{50} of aqueous extract and methanol in 24.77 µg mL^{-1} and 9.97 µg mL^{-1}, respectively. Overall, *Syzygium* sp. is well documented as a natural antioxidant in ancient civilizations like Thai in Thailand where they used seeds as antioxidants (Maisuthisakul et al., 2007). *Syzygium* sp. species are reported to be very rich in

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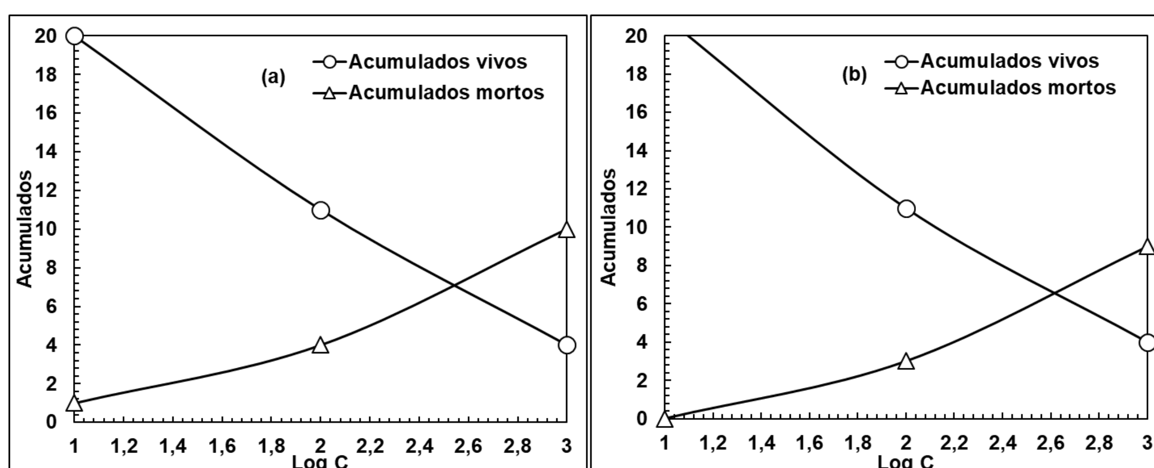
tannins, flavonoids, EOs, anthocyanins and other phenolic constituents (Reynertson et al., 2008; Sharma et al., 2003) . Fifteen polyphenols and two acylated flavonal glycosides have been isolated from leave *S. cumini* (L) Skeels (Mahmoud et al., 2001).

Antioxidant agents reduce oxidative stress one of the main mechanisms involved in the pathogenesis and progression of chronic diseases such as cancer, cardiovascular and inflammatory diseases (Krishnaiah et al., 2011). In this sense, the indications for popular use of *H. courbaril* bark to treat inflammatory processes, ulcers, arthritis and rheumatism (Fernandes et al., 2015; Jayaprakasam et al., 2007) seem to be linked to its antioxidant potential and its chemical constitution.

3.4. Toxicity

Lethality tests are performed in toxicological tests and the median lethal concentration (LC_{50}) can be obtained, which indicates death in half of a sample (Bednarczuk et al., 2010). *Artemia salina* is a microcrustacean used in fish feeding and is widely used in toxicological studies due to low cost and easy cultivation. Figure 3 shows the concentration log graph versus the number accumulated alive and dead for action of the EOs front *Artemia salina*, according to the Reed & Muench method (Reed&Muench, 1938).

Figure 3. Log graph of concentration versus the accumulated *Artemia salina* do (a) EO de *H. courbaril* (b) EO de *S. cumini.*



Source: Authors (2020).

Table 5 presents the LC_{50} obtained for the action of the EOs against *Artemia salina*.

Table 5. LC_{50} for action of the EOs front *Artemia salina*.

EO	LC_{50} mg L^{-1}	Classification
H. courbaril	354,80 ± 2,02	Nontoxic
S. cumini	412,10 ± 5,25	Nontoxic

Source: Authors (2020).

It was observed that both EOs were classified as nontoxic. Bitencourt et al. (2016) also reported that no acute toxicity was observed in the aqueous extracts of *S. cumini* seeds in the *Artemia salina* lethality assay and in rats. Dias et al. (2013) evaluating toxicity to non-target organisms, concluded that EO showed an LC_{50} of 175 mg L^{-1}. Krishnaraju et al. (2006) analyzing the extract of *S. cumini* seeds observed the LC_{50} of 475 mg L^{-1}, confirming the nontoxic potential observed.

On the other hand, the EO of *H. courbaril* brings satisfactory results when confronted with the literature, since Pereira et al. (2007) using the EO of *H. courbaril* resin obtained a LC_{50} of 8.83 mg L^{-1}. Fonseca et al. (2002) using the extract of *H. courbaril* seeds observed its toxicity. Thus, the EO of the bark brings an innovation in the aspect of use and application potential that becomes quite high.

Toxicity studies, as in this study, are conducted as a way to evaluate or predict toxic effects on biological systems and the relative toxicity of compounds to the environment, through a previous ecotoxicology assay on living organisms (Silva et al., 2016). Several species should be used as test organisms, such as the microcrustacean *Artemia* sp., fish and plants. Because it is relatively simple in the laboratory and widely distributed, *Artemia* sp. has been widely used as an indicator in acute toxicity tests (Silva et al., 2016). Therefore, this bioassay occurs quickly, with low economic cost and high reproducibility (Nunes et al., 2006). The toxicity of plants used in traditional medicine is cited by Miao et al. (2009). Toxicity in molluscicide s and larvicidal agents is explained by Luna et al. (2005) and insecticide by Uchida et al. (1965). The tests on *Artemia salina* are also used to express the safety of EOs or products of plant origin with molluscicidal activity, as it indicates the possibility of toxicity against non-target organisms, such as fish and small crustaceans, which occur in the same place as the snails(Carvalho, 2018). Although natural molluscicides are biodegradable, at certain concentrations, within the values required by who, their derivatives

may present risks. For this reason, it is necessary to perform tests that evaluate the toxicities of potentially molluscicide plants(Santos et al., 2007; Luna et al., 2005).

3.5. Molluscicidal activity

The death of the snails by molluscicide was evidenced by the retraction of the cephalopodal mass into the shell, release of hemolymph or a swelling with consequent prolongation of its cephalopodal mass out of the shell due to the breakdown of osmotic balance that is under neurohormonal control (McCullough et al., 1980). The results obtained for action of the EOs are presented in Table 6 for *H. courbaril* and Table 7 for *S. cumini*.

Table 6. Mortality of *Biomphalaria glabrata* in the face of the EO action of *H. courbaril.*

Exposição (h)	Concentração (mg $L^{-1)}$	Mortalidade (%)	LC_{50} mg L^{-1}	LC_{90} mg L^{-1}	R^2
24h	80 mg L^{-1}	95.33	37.34 ± (33.15-41.52)	73.24 ± (69.05-77.42)	0,8567
	60 mg L^{-1}	75.00			
	40 mg L^{-1}	65.33			
	20 mg L^{-1}	10.33			
	10 mg L^{-1}	30.00			
48h	80 mg L^{-1}	100.00	25.79 ± (21.70-29.88)	68.82 ± (64.73-72.91)	0,9775
	60 mg L^{-1}	80.00			
	40 mg L^{-1}	65.33			
	20 mg L^{-1}	50.00			
	10 mg L^{-1}	30.00			
72h	80 mg L^{-1}	100.00	24.74 ± (20.67-28.81)	67.90 ± (63.83-71.97)	0,9649
	60 mg L^{-1}	80.00			
	40 mg L^{-1}	70.00			
	20 mg L^{-1}	50.00			
	10 mg L^{-1}	30.00			

Source: Authors (2020).

Table 6 shows mortality of snails of 95.33 and 100% in 24 and 48 h, respectively.

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Table 7. Mortality of *Biomphalaria glabrata* snails in the face of The EO action of *S. cumini.*

Exposure (h)	Concentration (mg L^{-1})	Mortality (%)	LC_{50} mg L^{-1}	LC_{90} mg L^{-1}	R^2
24h	80 mg L^{-1}	90.00	44.76 ± (40.54-48.97)	77.20 ± (72.99-81.42)	0,9660
	60 mg L^{-1}	75.00			
	40 mg L^{-1}	45.00			
	20 mg L^{-1}	10.00			
	10 mg L^{-1}	13.00			
48h	80 mg L^{-1}	95.33	42.20 ± (38.04-46.36)	72.48 ± (68.32-76.65)	0,9629
	60 mg L^{-1}	80.33			
	40 mg L^{-1}	50.00			
	20 mg L^{-1}	10.00			
	10 mg L^{-1}	13.00			
72h	80 mg L^{-1}	95.33	39.21 ± (35.04-43.38)	72.71 ± (68.54-76.89)	0,9907
	60 mg L^{-1}	80.33			
	40 mg L^{-1}	50.00			
	20 mg L^{-1}	26.00			
	10 mg L^{-1}	15.00			

Source: Authors (2020).

Table 7 shows mortality of 90.00 and 95.33% snails in 24 and 48 h, respectively. According to WHO (1983), a plant with molluscicide property is considered active if it causes 90% mortality, at concentrations of up to 100 mg L^{-1}. Thus, both EOs fit into who's criteria. For a substance to be considered molluscicide, it must eliminate the snail at all stages of its life cycle and its natural habitat, at low concentrations, low cost, be stable in tropical conditions, ease of 22 application and transport, have selective lethality to the snail, present no risks to man and nor the surrounding fauna and flora, do not suffer decomposition in water and soil and be stable in conditions of temperature and solar irradiation (Hlth, 1965; WHO, 1983, 2002).

The results presented for *S. cumini* become satisfactory when compared to those of Dias et al. (2013) who evaluated the molluscicidal effect of *S. cumini* EO against

Biomphalaria glabrata and the LC obtained was 90 mg L^{-1}, and thus the 90% mortality rate is above 100 mg L^{-1}, outside the criteria of (Dolabela, 1997).

Comparing the LC_{90} of 73.24 mg L^{-1} obtained in this study to LC_{90} of 100 mg L^{-1} reported by Melendez & Capriles (2006) when evaluating the action of extracts of *H. courbaril* against *Biomphalaria glabrata*, the results presented in this study were extremely satisfactory. Numerous molluscicide substances have been isolated from several plant species and studies suggest that such biological activity is related to the presence of secondary metabolites such as flavonoids, alkaloids, terpenoids, saponins, tannins, steroids, among several others. It is necessary to understand the mechanism of action of these compounds on molluscs. The phytochemical profile of molluscicide helps clarify this process, as well as the physiological response of the snails forward to these chemical constituents. However, although several plants have been tested as possible molluscicide agents, little is known about their active metabolites (Cantanhede et al., 2010; Costa Lopes et al., 2011; WHO, 1983). The use of natural products has been widely studied in recent times with the purpose of obtaining bioactive substances that are safe and effective. There is economic interest in such products, which generates stimulus in researchers, research institutions and technological development for the characterization and study of their properties. In this context, EOs stand out (Simões et al., 2016).

4. Final considerations

The results allowed to confirm that the use of EO has relevant biological action, and its potential application as natural molluscicide was observed and strongly encouraged in this study.

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