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Chemistry

Chemical characterization, toxicity, antioxidant and antimicrobial activity of the essential oils of *Hymenaea courbaril* L. and *Syzygium cumini* (L.) Skeels

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ABSTRACT

This study evaluated the antimicrobial, antioxidant and toxicity activity of essential oils (EOs) of *Hymenaea courbaril* L. var. courbaril bark and *Syzygium cumini* (L.) Skeels leaves. The EOs were extracted by hydrodistillation and chemically characterized by gas chromatography coupled to mass spectrometry (GC/MS). The ABTS and DPPH assay were used to evaluate antioxidant activity. For the toxicity assay, lethality was evaluated against Artemia salina Leach. For the antimicrobial assay, the method of Disc Diffusion and Dilution in Broth was applied to obtain the minimum inhibitory concentration and minimum bactericidal. The major constituent of the EO of H. courbaril was β-ocimene (23.33%) and the EO of S. cumini was isocaryophyllene (18.01%). Both OE showed relevant antioxidant activity and low toxicity against Artemia salina. The EOs showed bactericidal activity against E. coli, S. aureus, P. aeruginosa, Salmonella sp., B. cereus and P. mirabilis. The results obtained are encouraged by the potential use of the OE's studied in the control and fight of pathogenic microorganisms.

Keywords: Essential oil; Antimicrobial; Toxicity; Hymenaea courbaril; Syzygium cumini

RESUMO

Este estudo avaliou a atividade antimicrobiana, antioxidante e toxicidade de óleos essenciais (OE's) das cascas de *Hymenaea courbaril* L. var. courbaril e folhas de *Syzygium cumini* (L.) Skeels. Os OE's foram extraídos por hidrodestilação e caracterizados quimicamente por cromatografia gasosa acoplada à espectrometria de massa (CG/EM). O ensaio ABTS e DPPH foram utilizados para avaliar a atividade antioxidante. Para o ensaio de toxicidade, a letalidade foi avaliada frente a Artemia salina Leach. Para o

ensaio antimicrobiano, o método de Difusão de Disco e Diluição no Caldo foram aplicados para obter a concentração inibidora mínima e bactericida mínima. O principal constituinte do OE de H. courbaril foi β-ocimeno (23,33%) e o OE de S. cumini foi isocariofileno (18,01%). Ambos os OE apresentaram atividade antioxidante relevante e baixa toxicidade frente a Artemia salina. Os OE's apresentaram atividade bactericida contra E. coli, S. aureus, P. aeruginosa, Salmonella sp., B. cereus e P. mirabilis. Os resultados obtidos são encorajados pelo potencial uso dos OE's estudados no controle e combate de microrganismos patogênicos.

Palavras-chave: Óleo essencial; Antimicrobiano; Toxicidade; Hymenaea courbaril; Syzygium cumini

1 INTRODUCTION

The use of plants for therapeutic purposes is an ancient practice carried out by human civilization over centuries, based on random discoveries, where products of mineral, vegetable and animal origin were used, these being the main sources of drugs (VARGAS *et al.*, 2019). The properties of medicinal plants are directly related to their essential oils (EOs), which according to are components that integrate the secondary metabolites of plants, that is, they are part of the non-primal system of these organisms, having functions of protection against elements external to plants (SIMÕES, 2010).

Hymenaea courbaril and *Syzygium cumini* stand out among medicinal plants with efficient biological potentials. The *H. courbaril* of the Fabaceae family, which has as popular name jatobá, jatobazeiro or jatobá-verdadeiro. A native, semi-deciduous species, belonging to the Atlantic Forest biome and occasionally also to the Cerrado, with wide distribution in Brazil, mainly in Piauí and northern Paraná (DUARTE *et al.*, 2016).

In traditional medicine, the use of infusions and decoctions of the stem bark of *Hymenaea courbaril* is widely indicated, however reports of use of leaves, roots and fruits are also cited (BEZERRA *et al.*, 2013). Tea obtained from the stems of *H. courbaril* is popularly used as analgesic, antiseptic, expectorant, laxative, purgative, sedative, stimulant and tonic, to treat ulcers, inflammations, arthritis and rheumatism (FERNANDES *et al.*, 2015). In addition to studies in relation to the EO

of the leaves of the *H. courbaril* tree that present antimicrobial potential and antioxidant activity of phenolic compounds present in this plant. Therefore, an important aspect associated with *H. courbaril* is the presence of these products in its constitution (VEGGI *et al.*, 2014). In this sense, *H. courbaril*, is a kind of popular use, which presents in its constitution biologically active compounds, and few scientific studies. Fruit peels are often discarded due to this was the object of this study.

S. cumini which is a plant of the Family Myrtaceae, originating in Eastern India, and its leaves are rich in tannins and saponins (PEREIRA *et al.*, 2016), having proven physiological effect in several species, including man (GOULART *et al.*, 2016). Despite this abundance, these fruits are not traditionally consumed. Several studies have demonstrated the pharmacological and biological activities of *S. cumini* such as antidiabetic (SALES *et al.*, 2019; TONG *et al.*, 2014), anti-inflammatory (RIBEIRO *et. al.*, 2014) and antimicrobial (XAVIER, 2015; AYYANAR; SUBASH-BABU, 2012). This natural compound has aroused interest due to its nutritional and therapeutic effects, mainly due to its antioxidant action. The use of seed powder stands out for its hypoglycemic, antibacterial, antifungal and anti-diarrhoeal actions (XAVIER, 2015).

The emerging spread of antimicrobial-resistant bacterial strains is a serious global public health problem and despite the great advance in the development of new broad-spectrum antimicrobials, the indiscriminate use of these drugs has selected resistant microbial populations, thus limiting the therapeutic options of infectious processes (FERREIRA *et al.*, 2010).

In the context of microbial resistance, gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria stand out. *S. aureus* stands out for being one of the most important pathogens, as it acts as an infectious agent causing a

series of infections, ranging from localized infections such as abscesses, to disseminated infections such as severe septicemia (ALVES *et al.*, 2016) and E. coli is the most important causative agent of urinary tract infections, which over the years has shown acquired resistance to the main drugs used in empirical therapy of this type of infection, such as fluoroquinolones (MATTOS *et al.*, 2014).

In the studies that aimed to evaluate the epidemiological profile of cases of outbreaks of diseases transmitted by ingestion of contaminated food, they observed that the most significant responsible agents in the positive samples were Gram-negative bacteria, mainly *E. coli* and *Salmonella sp*. (ALMEIDA *et al.*, 2013). *Salmonella sp*. was the pathogen that was most related to outbreaks of diseases transmitted by contaminated food, basing on the realization of its wide distribution in nature, and may be present in water, animals and humans (MARQUES *et al.*, 2013).

S. aureus is an important pathogen associated with nosocomial diseases acquired in the community. Although they may be present in various places of the body, the nasopharynx and oropharynx are the most frequent sites (KLEIN *et al.*, 2013). *S. aureus* has the ability to asymptomatically colonize normal people. It is estimated that about 20 to 40% of the world population are asymptomatic nasal carriers of *S. aureus* and *E. coli* and with this are at higher risk of infection (NIMMO *et al.*, 2013). Currently, it is almost impossible to speak of hospital infection and not to report *P. aeruginosa*. For, in the last four decades, this microorganism has accounted for 10% of all cases of nosocomial infections reported (NTZIMANI *et al.*, 2010). Thus, epidemiological studies help in the monitoring of microorganisms of great pathogenic potential.

Approximately 25% of the drugs prescribed worldwide for the treatment of these infections are derived from plants and of the 252 drugs considered basic and essential by the World Health Organization (WHO) 11% are exclusively originated

from plants and a significant number are synthetic drugs obtained from natural precursors (SIMÕES *et al.*, 2016). In Brazil, with the purpose of improving the health service provided to the population, the Unified Health System (UHS) together with the World Health Organization (WHO), recognizes the use of medicinal plants for therapeutic purposes, and ensures the use of these herbal medicines in primary health care (BRASIL, 2011).

In view of the above, the present study evaluated the chemical profile, antioxidant and antimicrobial action potential of The EO of the barks of *H. courbaril* (jatobá) and Leaves *Syzygium cumini* (jambolão), applying chemical and physicochemical methodologies, in order to establish the appropriate conditions of stability and aiming to find alternatives in controlof the dissemination of antimicrobial resistance and development of new drugs effective in combating them.

2 MATERIAL AND METHODS

2.1 Plant material

The collection of plant material used in this research was carried out in October to December 2019. The collection period was based on previous studies that proved better action of the OEs of S. cumini and H. courbaril when collected in the months between October and December compared to those collected between January and September. They were identified by the Herbário Ático Seabra of the Federal University of Maranhão and a sample of each plant was deposited. Leaves of Syzygium cumini (L.) Skeels (record no. 1069) were collected in São Luís (MA) and fruit peels of Hymenaea courbaril L. var. courbaril (record no. 1079) in Palmeirândia (MA). After collection, the plant species were transported to the Laboratory of Research and Application of Essential Oils (LOEPAV-UFMA), where they were submitted to the convective air-drying oven at 45 °C for 24 hours, and subsequently crushed in a knife mill.

2.2 Obtaining the EO

The plant materials were collected and transported to the Laboratory of Research and Application of Essential Oils (LOEPAV-UFMA), where they were crushed and stored for extraction of the EO. For the extraction of the EO, 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. 120g of *H. courbaril* and 200g of *S. cumini* was 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 the samples stored in amber glass ampoules under 4°C refrigeration obtained an average yield of 0.46%. Subsequently submitted the analyses.

2.3 Analyses of chemical constituents

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 validated 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 Total phenolics

The determination of the total phenolic compounds of the EO was performed with adaptation of the Folin-Ciocalteu method (WATERHOUSE, 2002). We used 5 mg of EO diluted in 1 mL of ethanol. 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.

2.5 Antioxidant activity by the ABTS method

The determination of antioxidant activity was performed by the ABTS method [2,2-azinobis-(3- ethylbenzotiazolin-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 absorption from 0.7±0.01 to 734 nm.

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 EO concentration 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 absorption of the reaction mixture was performed in a spectrophotometer of 734 nm.

The capture of the free radical was expressed as a percentage of inhibition (%I) of the radical action ABTS according to Equation 1 (BABILI *et al.*, 2011), where Abs_{ABTS} represents the absorption of the radical solution ABTS and ABSAM represents the absorption of the sample.

$$\%InhibitionABTS = (ABS_{ABTS} - ABS_{AM})/ABS_{ABTS} \quad (1)$$

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

2.6 Antioxidant activity by the DPPH method

To determine the antioxidant capacity by the DPPH method of the EO, the adapted methodology of Brand-Williams et al. (1995) was used. The radical was prepared by dissolving 3.94 mg of DPPH (2,2-Diphenyl-1-picrylhydrazyl) in 100 mL of ethanol. From the concentrations of the EOs (5 to 150 µg mL^{-1}) the reaction mixture with the radical cation ABTS was prepared. 50 µL of EO was mixed with 950 µL of ethanol, 2 mL of DPPH radical solution, and completed up to 4 mL with ethanol, and again homogenized. The mixture was left to react in the dark for 30 minutes and the absorption of the reaction mixture was performed in a spectrophotometer of 517 nm.

The capture of the free radical was expressed as a percentage of inhibition (%I) of the action of the DPPH radical according to Equation 2 adapted by Babili et al. (2011), where Abs_{DPPH} represents the absorption of the radical solution DPPH and ABS_{DPPH} represents the absorption of the sample.

$$\%InhibitionDPPH = (ABS_{DPPH} - ABS_{AM})/ABS_{DPPH} \quad (2)$$

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

2.7 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 solutions (60 g of sea salt/1L of distilled water) were added. 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, given that they did not cross the partition. The part of the system containing Artemia salina 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 to homogenize the physical conditions of the test organisms. Incubation was performed for a period of 48 h. 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.

To quantify the efficiency of the EO, the Reed & Muench statistical test (1938) was applied, calculated by Pizzi (1950). The criterion established by Dolabela (1997) was adopted for classification of EO toxicity, being considered highly toxic when $LC_{50} \leq 80$ mg L^{-1}, moderately toxic 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}.

2.8 Standardization of microbial inoculum for sensitivity tests

Bacteria strains were used: *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 15442, *Bacillus cereus* (ATCC 11778), *Proteus mirabilis* (ATCC 25933) and *Salmonella sp.* (ATCC 700623). These were previously identified and confirmed by biochemical tests.

Pure microbial cultures maintained in TSA Agar were peaked for brain and heart infusion broth (BHI) and incubated at 35 °C until they reached exponential growth phase (4-6 h). After this period, the cultures had their cell density adjusted in 0.85% sterile saline solution, in order to obtain turbidity comparable to that of the standard McFarland solution 0.5, which results in a microbial suspension containing approximately 1.5 x 108 CFU mL^{-1} according to the standards of the Clinical and Laboratory Standards Institute (2020).

2.9 Disk Diffusion Method

Antimicrobial activity was performed according to the disc diffusion technique of the Clinical and Laboratory Standards Institute (2020), which standardizes the sensitivity tests of antimicrobials by disc-diffusion, using standardized suspensions of the strains distributed in plates containing Mueller Hinton Agar culture medium (AMH) plus discs containing 20 µL of EO. Gentamicin (30 µg) was used as a positive control. The plates were incubated in a bacteriological greenhouse at 35 °C/24 h. The diameters of the inhibition halos were measured, including the diameter of the disc. These trials were done in triplicate.

2.10 Inhibitory Concentration and Minimal Bactericidal

To determine the Minimum Inhibitory Concentration (MIC), the broth dilution technique was used. With serial dilutions of EO in Mueller Hinton Broth (MH), resulting in concentrations of 1000, 500, 250, 100, 50, 25, 10 and 5 µg mL^{-1}, performing sterility controls and incubation at 35ºC for 24 hours. After the incubation period, the minimum inhibitory concentration of EO was verified, being defined as the lowest concentration that visibly inhibited bacterial growth (absence of visible cloudiness). Tests performed in triplicate. The Minimum Bactericidal

Concentration (MBC) was measured from the inoculation of 10 µL of the tubes resulting from the dilution in Mueller Hinton Broth, performed a plate count after 24h, where the plates that did not grow colonies were classified as bactericidal concentrations for the action of EO or EH.

3 RESULTS AND DISCUSSION

3.1 Chemical constituents

GC/MS allowed the chemical characterization of the EOs and a Figure 1 shows the EO chromatogram of *H. courbaril* where 27 peaks are displayed.

Figure 6 – *H. courbaril* EO chromatogram

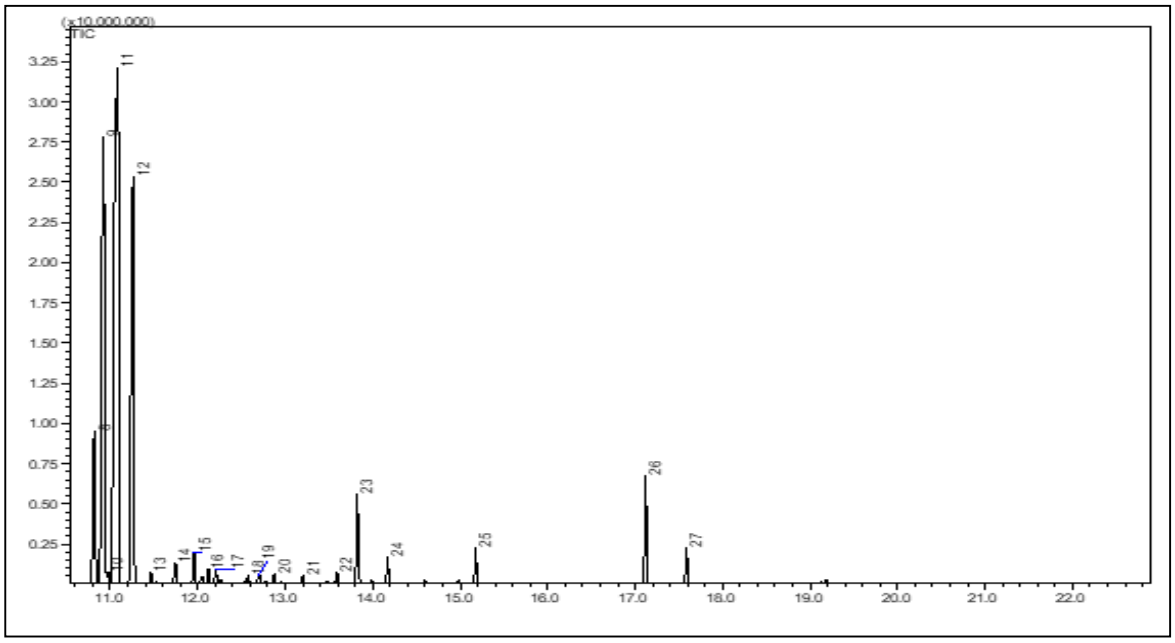


Table 1 shows the chemical constituents obtained in the EO *H. courbaril* sample. 25 constituents were identified and the majority were: β-ocimene (23.33%), d-limonene (13.51%), α-pinene (12.33%), β-pinene (11.79%) and β-myrcene (11,38%).

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

Pico	RT	Constituintes NIST08	Classe	%
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	Canfene	Monoterpene	4,31
5	9,843	β-pinene	Monoterpene	11,79
6	10,118	β-myrcene	Monoterpene	11,38
7	10,427	(E) 3-hexen-1-ol	Monoterpene	0,08
8	10,816	Ocimene	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-noneno-3-ino	Monoterpene	0,20
20	12,875	Oxirano	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	acetato de fenila	Monoterpene	0,48
25	15,179	borneol	Monoterpene	0,63
26/27	-	Unidentified	-	1,46

Discordant results were observed by Aguiar *et al.* (2010) verified the chromatographic analyses performed in GC/MS of the EO of the ripe and green fruit peels of *H. courbaril*, it was possible to detect 47 compounds, representing 86.1 % of the constituents of the EO. The major components of The EO of ripe fruit

peels were: α-copaene (11.1%), sphatulenol (10.1%), β-selinene (8.2%), γ-muurolene (7.9%) and buckylene oxide (6.9%). However, the EO obtained from the peels of the green fruits of *H. courbaril* presented the major components: germacrene-D (31.9%), β-caryophyllene (27.1%), bicyclogermacreno (6.5%), α-humulene (4.2%) and α-copaene (4.2%).

Mercês *et al.* (2018) evaluated the chemical composition of the EO of *H. courbaril* by CG/MS, 26 compounds were identified, representing 76.03% of the constituents of the EO. The analysis revealed the hydrophyllene oxide and β-caryophyllene as the majority compounds of this EO with 20.63% and 16.78%, respectively. Costa *et al.* (2017) evaluated the composition of EO of ripe fruit peels and leaves of *H. courbaril*, identified the following major components: α-copaene, spathulenol and β-selinene (11.1%, 10.1% and 8.2%, respectively), which were present in leaves in small concentrations in EO of leaves of *H. courbaril*.

According to Lima *et al.* (2005) α-pinene and β-pinene monoterpenes demonstrate satisfactory antifungal activity of the components, as well as Gundidza *et al.* (2008) that prove the antimicrobial action of this EO on *Escherichia coli*, *Clostridium perfringens* and *Aspergillus flavus*, the antimicrobial activity found is associated with the high concentration of the monoterpene. Guerra *et al.* (2014) reported that β-ocimene is one of the major components of EO of *Schinus terebenthifolius* leaves. This monoterpene has antimicrobial activity against *Staphylococcus aureus* strains. Demonstrating a strong promise in the fight against pathogenic microorganisms. Figure 2 shows the EO chromatogram of *S. cumini* where 28 peaks are displayed.

Figure 2 – Cromatograma do EO de *S. cumini*

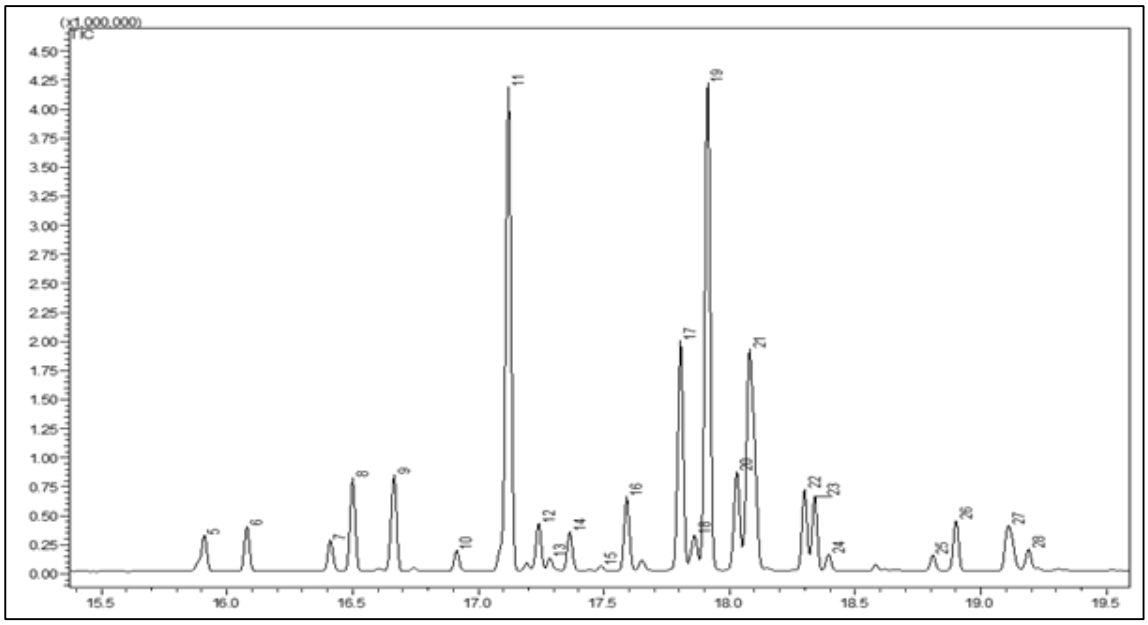


Table 2 shows the chemical constituents obtained from the EO S. cumini sample. 28 constituents were identified and the majority were: isocaryophyllene (18.01%), naphthalene (17.37%) and longifolene (11.65%).

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

Order	RT (min)	Constituents NIST08	Class	%T
1	7,523	1-(1-metil-2-ciclopenten-1-il) - etanone	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-cubebene	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	Isocaryophyllene	Sesquisterpene	18,01
12	17,240	Sesquiterpene	Sesquisterpene	1,68

Continuation...

Conclusion				
Order	**RT (min)**	**Constituents NIST08**	**Class**	**%T**
13	17,285	α-guaiene	Sesquisterpene	0,49
14	17,365	Spathulenol	Sesquisterpene	1,40
15	17,489	isogermacrene D	Sesquisterpene	0,22
16	17,591	α-humulene	Sesquisterpene	2,62
17	17,805	γ-cadinene	Sesquisterpene	8,28
18	17,860	naphtaleno (isômero)	Monoterpene	1,44
19	17,913	naphtaleno (isômero)	Monoterpene	17,37
20	18,029	Virdiflorene	Sesquisterpene	3,79
21	18,080	longifolene (V4)	Sesquisterpene	11,65
22	18,298	(+) - δ-cadinene	Sesquisterpene	2,79
23	18,339	(+) - δ-cadinene (isomer)	Sesquisterpene	2,41
24	18,394	Calamenene	Sesquisterpene	0,59
25	18,810	Nerolidol	Sesquisterpene	0,54
26	18,901	γ-elemene	Sesquisterpene	1,78
27	19,110	dietil ftalato	Monoterpene	2,35
28	19,190	caryophyllene oxide	Sesquisterpene	0,66

Discordant results were observed by Mohamed *et al.* (2013) evaluated the chemical composition of EO of *S. cumini* leaves through GC-MS analysis, identified 49 chemical components representing about 98.3% of the EOs. The abundant constituents of the oil were: α-pinene (32.32%), β-pinene (12.44%), trans-caryophyllene (11.19%), 1, 3, 6-octatene (8.41%), delta-3-carene (5.55%), α-caryophyllene (4.36%) and α-limonene (3.42%).

Machado *et al.* (2013) identified 12 chemical compounds of EO from *S. cumini* leaves, by CG-MS method. The major components were: α-caylophyllene (25.24%), β-caylophyllene (16.00%) and α-terpine (9.08%). Most of the constituents found are sesquiterpenes such as α-cayophyllene and β-cariophyllene, their alcohol and epoxide, as well as terpineol. Compounds found in this work in smaller compositions.

Dias et al. (2013) evaluated the chemical composition of the EO of jambolão leaves, found 11 chemical compounds present, reaching about 99.98% of the total constitution, the major components verified in this EO are: α-pinene (31.85%), (Z)-

β-ocimeno (28.98%) and (E)-β-ocimeno (11.71%). Brito *et al.* (2007) analyzed assiduity of the compounds present and characterized that α-pinene and ocimen isomers are main constituents of EOs isolated from leaves, stems and fruits of *S. cumini.*

Nishandhini *et al.* (2015) analyzed the chemical composition of EO of the pulp of green fruits and leaves of *S. cumini*, using cg-ms and CG-FID (Gas Chromatography - Flame Ionization Detector) techniques. They identified 34 chemical components representing 99.3% of green fruit pulp oil and 66 components constituting 95.3% of leaf EO. The main components of EO of green fruit pulp were: α-pinene (12.4%), β-pinene (8.0%), myrcene (8.4%), α-terpinessential (7.4%), δ-cadinene (7.7%) and α-cadinol (25.8%). However, the main components of leaf EO were: α-pinene (21.5%), trans-ocimen (6.8%), a-terpinessential oil (9.5%) and δ-cadine (8.3%).

Yang-Jiang *et al.* (2011) analyzed the major components of Rosmarinus officinalis EO, known as rosemary. They identified that the major components were: 1.8-Cineol (26.54%) and alpha-pinene (20.14%). Making it possible to conclude that α-pinene bought antimicrobial activity on the strains of *S. epidermidis*, *S. aureus*, *B. subtilis*, *P. vulgaris*, *P. aeruginosa*, *E. coli*, *C. albicans* and *A. niger*.

Celedonio *et al.* (2008) reported the peripheral antinociceptive effect and antiedematous activity of α-pinene and Croton argyrophylloides EO, which is the major compound of this EO. Garcia *et al.* (2008) evaluated the action of α-pinene and citral monoterpenes, in which they obtained as results an excellent fungicide activity when placed in front of the fungi *Colletotrichum musae*, *Colletotrichum gloeosporioides* and *Fusarium subglutinans* f.sp ananás.

3.2 Total phenolics

The amount of total phenolics found in the EO of *H. courbaril* L was 490.353 mg EAT g^{-1}. Similar behavior was reported in studies by Vencato *et al.* (2016) and Veggi *et al.* (2014) for The EO of *H. courbaril* bark the values found were respectively 516.89 and 335.0 mg EAT g^{-1}. According to Table 4, the total phenolic content for EO of *Syzygium cumini* (L.) was 578,453 mg EAT g^{-1}. In previous studies Veber et al. (2015) and Santos (2017) determined the presence of total phenolic compounds in leaves of *Syzygium cumini* (L.) by different methods, where values ranged from

458.33 to 986.11 EAT g^{-1} were found. Thus, showing that the results found in this study are in accordance with the literature.

The peels of the fruit of *H. courbaril* L and the leaves *Syzygium cumini* (L.) presented in this study significant amounts of total phenols. Variable levels of phenolic compounds are naturally produced as secondary metabolites in vegetables. Phenolic quantitative may oscillate according to the physiological need of the plant, being some reasons linked to physiological stress, attack by phytopathogens, insects or herbivore animals (Oliveira *et al.* 2014).

Phenolic compounds present as one of the chemical groups, flavonoids that present important antioxidant action (SILVA *et al.*, 2010). These compounds include simple molecules and also molecules with high polymerization, present in vegetables in free form or linked to sugars or proteins. More than 8000 different types have already been found in plants and are classified as little or highly distributed in nature. Among those of little distribution are simple phenols, 33 pyrocatechol, hydroquinone, resorcinol, tannins, lignin and also aldehydes derived from benzoic acids that are present in some essential oils. In the group of highly distributed are flavonoids and derivatives and phenolic and coumarin acids (SILVA, 2010).

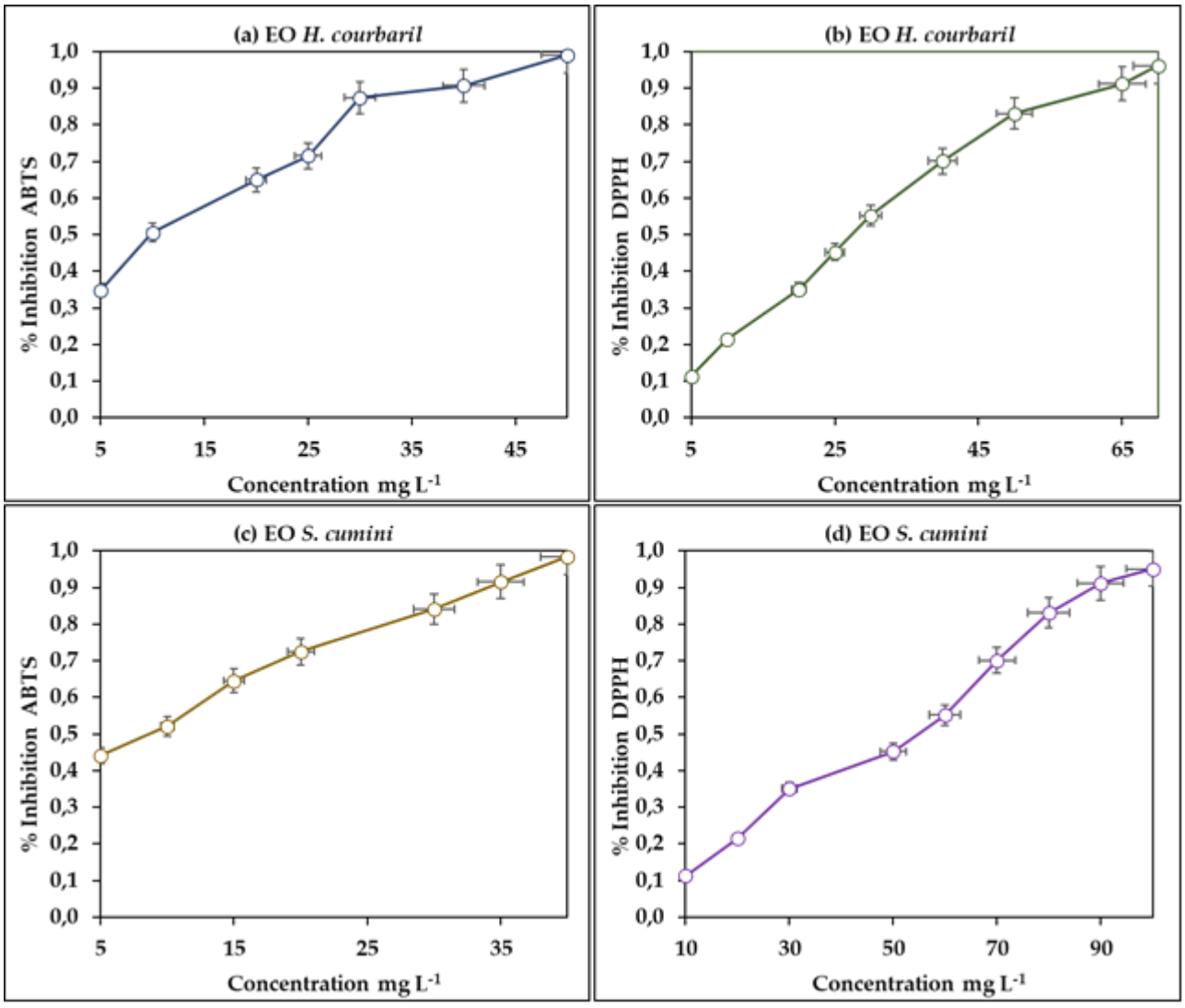
These compounds act as antioxidants, because in addition to providing electrons or hydrogen, there is also the formation of stable intermediate radicals during the reaction with free radicals. They are characterized as molecules that have a benzene ring linked to one or more hydroxyl groups (SILVA *et al.*, 2010; JARDINI, 2010).

3.3 Antioxidant activity

Figure 8 shows the graphs that relate the concentration of EOs in mg L^{-1} and the percentage of inhibition of the radical ABTS and DPPH. The equations of the lines obtained using the ABTS test were y = 0.0140x + 0.352 (R^2 = 0.9802) for the EO of *H. courbaril*; y = 0.0153x + 0.3872 (R^2 =0.9877) for The EO of *S. cumini*. For the DPPH test, the equations of the lines were y=0.0131x+0.1067 (R^2= 0.9690);

y=0.0096+0.0208 (R2=0.9871) for *H. courbaril* and *S. cumini*, respectively. From these equations, the respective values of the effective concentration or IC_{50} and IC_{90} were calculated.

Figure 3 – Percentage inhibition versus concentration of EOs (a) ABTS EO *H. courbaril* (b) DPPH EO H. courbaril (c) ABTS EO *S. cumini* (d) DPPH EO *S. cumini*



The results of the calculations of antioxidant potentials of The EO of *H. courbaril* and *S. cumini*, shown in Table 3, were interpreted based on the efficient concentration IC_{50} and IC_{90}, in mg L^{-1}. Table 3 presents the results obtained for the antioxidant capacity of the EOs.

Table 3 – Antioxidant capacity of EOs

EO	Method	IC_{50} mg L^{-1}	IC_{90} mg L^{-1}
H. courbaril	ABTS	10,57	39,14
S. cumini		7,37	33,52
H. courbaril	DPPH	30,02	60,56
S. cumini		49,92	91,58

According to Sousa *et al.* (2011) and Mishra *et al.* (2012), the lower the CI50 value, the higher the antioxidant activity of the plant compound, since lower oil concentration is needed to reduce the DPPH and ABTS radical by 50%. Thus, when analyzing Table 5, it was verified that the essential oil of S. cumini showed better antioxidant activity by the ABTS method, in relation to the EO of *H. courbaril*.

Sousa *et al.* (2016) also found antioxidant activity according to the ABTS methodology for S. cumini extract, with a value of 43.67 ±0.2 expressed in trolox g^{-1} µmol. Almada (2013) also evaluated the antioxidant activity of the species of *S. cumini*, and found CI50 of 2,148.88 ± 140.26 µmol of Trolox.g^{-1}. Miranda (2019) when evaluating the biological activity of *H. courbaril* and other plants, showed antioxidant capacity with IC_{50} of 201.7 µg mL^{-1}, being related to its phenol concentration.

According to the results found using the DPPH methodology, *H. courbaril* proved to be more efficient. These data are considerable, since Ucker *et al.* (2016) did not find antioxidant activity in the DPPH assay of The EO of *S. cumini* seeds. In a study conducted by Luzia & Jorge (2009) to evaluate the antioxidant activity of *S. cumini*, they revealed maximum inhibition of the extract with values of 94.98% and 118.66 µg mL^{-1}. Similarly, Figueiredo (2014) demonstrated that the hydroethanolic extract of *H. courbaril* seed presented lower CI50 value among the analyzed samples (149.45µg mL^{-1}). Veggi *et al.* (2014), when using the extract of *H. courbaril*

obtained by extraction by supercritical fluid, presented a higher value of IC_{50} of 200µg mL^{-1}. Therefore, in this study it is possible to observe considerable antioxidant action of the EOs of *S. cumini* and *H. courbaril*, being subject to studies for use in the different industrial branches.

3.4 Toxicity

Table 4 describes the mortality data for three concentrations (1000, 100, 10 mg L-*1*), and its subsequent classification of the toxicity assay performed of The EOs of *H. courbaril* L. and *S. cumini* (L.) against *Artemia salina* L. The test result showed the relationship of organisms (living and dead) at the end of the assay.

Table 4 – Toxicity of EOs

EO	Concentration mg L^{-1}	% Mortality	LC_{50}	Classification
	1000	60		Nontoxic
H. courbaril	100	30	354,8 mg L^{-1}	(potential for encouraged
	10	10		application)
	1000	60		Nontoxic
S. cumini	100	25	398 mg L^{-1}	(potential for encouraged
	10	10		application)

The results of the *Artemia salina* test showed a decreasing trend in the percentage of survival, with the decrease in the concentration of the sample. It was observed that the highest mortality rate was found at the concentration of 1000 mg L^{-1} with 60% in 24 h. The analyzed EOs presented values of $LC_{50} \geq 250$ mg L^{-1}, thus indicating that it was nontoxic according to the reference methodology used for the test (DOLABELLA, 1997). Data on the toxicity of The EO of the bark of *H. courbaril* L against *Artemia salina* L. were not found in the scientific literature, emphasizing the importance of the findings of the present study.

The EO of *Syzygium cumini* (L.) did not present toxicity because it exhibited LC_{50} of 398 mg L^{-1}, a value higher than the reference to classify it as nontoxic, so this EO has its application potential acceptable and encouraged. In a study developed by Bitencourt et al. (2016), when using the leaves of *Syzygium cumini* (L.) showed a non-toxic effect against *Artemia salina* as well as George *et al.* (2017) who also found similar results, such data corroborate the results of this study.

The toxicity of a plant is considered to be the ability of the product studied to cause some imbalance, serious damage or even lead to death (LIMA *et al.*, 2014). Tests with natural products have revealed relevant biological activities. One trial that can demonstrate such activities is the bioassay using *Artemia salina*. There are numerous advantages to using mini crustaceans in scientific trials, the main ones include low cost, speed, easy maintenance and the use of a small sample (SAYFRIED[A12], 2017).

In vitro toxicity tests may be useful for the follow-up of action research of substances still unknown, as they provide preliminary data for evaluations in in vivo research, in order to elucidate the study of doses and the establishment of the dose-dependence correlation with the physiological effects manifested. In general, bioactive compounds are toxic to larvae of *Artemia salina* (BARCELOS *et al.*, 2017; MASSUD-FILHO *et al.*, 2016). Thus, lethality to this microcrustacean can be used as a quick and simple preliminary test during the isolation of natural products(COE *et al.*, 2010).

3.5 Antimicrobial activity

The results obtained with the essential oils of *H. courbaril* and *S. cumini* using the diffusion disc method are shown in Table 5. The antimicrobial activity of The EOs was performed against the bacteria *E. coli*, *S. aureus*, *P. aeruginosa*, *Salmonella*

sp., *B. cereus* and *P. mirabilis*. The same table also presents the minimum inhibitory concentrations (MICs) and minimum bactericides (MBCs), after performing the broth dilution technique.

Table 5 – Sensitivity of microbial strains for action of EOs in the face of the microorganisms tested

	EO *H. courbaril*			**EO *S. cumini***		
Species	DIH	MIC	MBC	DIH	MIC	MBC
E. coli	21	190	490	17	250	600
S. aureus	33	170	450	32	150	390
P. aeruginosa	11	800	1300	9	500	1000
Salmonella sp.	12	500	900	13	350	800
B. cereus	15	470	530	22	200	780
P. mirabilis	10	430	900	11	400	820

Note: DIH- average diameters of inhibition halos (mm); MIC- minimum inhibitory concentration (µg mL^{-1}); MBC- minimum bactericidal concentration (µg mL^{-1}); NI* - there was no inhibition of the microorganism by the essential oil tested.

According to the results observed, it is observed that the bacteria presented sensitivity to the EOs tested. A variation in inhibition halos covered between 9 and 33 mm (EO-sensitive strains) was detected. The EO of *H. courbaril* revealed antimicrobial activity against all tested strains, but exhibited a higher inhibition halo in the S. aureus strain (33 mm). These results are in accordance with the analyses pointed out by Sales (2014), who when testing the antimicrobial action of *H. courbaril* O against 8 microbial strains, pointed out sensitivity only to gram-positive bacterial strains of *S. aureus* at concentrations of 50 and 100 mg mL^{-1} of EO. Mercês *et al.* (2013), when performing a serial microdilution test, reported oil activity for all bacteria tested, including *S. aureus* and *E. coli*.

When analyzing *in vitro* the antimicrobial activity of three plant species, including *H. courbaril* on standard strains, Fernandes *et al.* (2005), observed that

jatobá hydroalcoholic extract inhibited about 60% of Gram-positive bacteria and did not present inhibition against Gram-negative bacteria species. According to the authors, *H. courbaril* has chemical compounds that may be associated with the antimicrobial potential of the plant.

However, Pereira (2007) found a higher antimicrobial potential on the gram-negative bacterium P. aeruginosa strain, a result contrary to that of this study. The same was presented by Verpoorte and Dihal (1987), in relation to the bark extract of *H. courbaril*, who obtained halo inhibition <15 mm under the concentration of 50 mg mL^{-1} with gram-negative strains of *Escherichia coli* and *Pseudomonas aeruginosa*.

In the present investigation, The EO of *S. cumini* was more effective especially against Gram-positive bacteria, such as *S. aureus* (32 mm) and *B. cereus* (22 mm). These results verify the relevance of this study, since Loguercio et al. (2005), when investigating the antimicrobial action of S. cumini extract, found lower inhibition halos than this study for these same bacteria. In the present investigation, the EO of *S. cumini* was more effective especially against Gram-positive bacteria, such as *S. aureus* (32 mm) and *B. cereus* (22 mm). These results verify the relevance of this study, since Loguercio et al. (2005), when investigating the antimicrobial action of *S. cumini* extract, found lower inhibition halos than this study for these same bacteria.

In this sense, Shafi *et al.* (2002) stated that the EO of *S. cumini* was more efficient in its antimicrobial action than *S. travancoricum*. Ucker *et al.* (2016), when evaluating the antimicrobial activity of EO of leaves and seeds of *S. cumini*, using another methodology for classifying antimicrobial action on substances, verified mean inhibition in relation to *S. aureus* and weak for other Gram-negative bacteria.

Most studies related to the investigation of the antibacterial action of EOs explain that they have lower sensitivity to Gram-negative bacteria. Thus, the

sensitivity rates compared to the isolates in this study can be explained by the fact that Gram-negative bacteria, such as *E. coli*, contain a cell wall with double protection separated by glycopeptide, which decreases the action of antimicrobials (Hirsh *et al.* 2003).

The MIC of the EOs followed the classification of Aligiannis *et al.* (2001), which proposes to classify plant products based on the results of the MIC. Strong inhibitors: MIC up to 500 μg mL^{-1}; moderate inhibitors: MIC between 600 and 1000 μg mL^{-1}; weak inhibitors: MIC above 1000 μg mL^{-1}.

The MIC of *H. courbaril* EO showed strong inhibition against five bacteria and moderate inhibition over *P. aeruginosa* (800 μg mL^{-1}). The EO of S. cumini showed strong inhibition on all bacterial strains tested. Thus, according to the results obtained, it is noted that the EO of *S. cumini* was more effective to inhibit the pathogenic bacteria tested.

However, Michelin *et al.* (2005) found that the extract of *S. cumini* did not inhibit standard strains of *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27583) and *S. aureus* (ATCC29213), demonstrating the importance of the results exhibited in this study with essential oils.

The results of the broth microdilution test performed by Martins *et al.* (2010) demonstrated the MIC of the crude extract of *H. courbaril* bark as a strong inhibitor, by presenting inhibition in the concentration of 350 μg mL^{-1} for the clinical isolates *S. aureus*, *E. coli* and *P. aeruginosa*.

The Tests of Minimum Bactericidal Concentrations (MBC) showed better results at concentrations of 450 and 390 μg mL^{-1} compared to *S. aureus*, for the EOs of *H. courbaril* and *S. cumini*, respectively, when compared the bactericidal action on *P. aeruginosa* which were only from 1300 μg mL^{-1} (*H. courbaril*) and 1000 μg mL^{-1} (*S. cumini*).

The authors Chandrasekaran & Venkatesalu (2004) tested extracts of *S. cumini* against Gram-positive bacteria (*B. subtilis, S. aureus*), Gram-negative (*S. typhimurium, P. aeruginosa, K. pneumoniae and E. coli*) and fungal strains. Bacterial activity showed that the *E. coli* strain presented a similar value (500 μg mL^{-1}) to that of this study and only two bacterial strains (*B. subtilis* and *S. a*ureus) had satisfactory bactericidal action.

In a study conducted by Fernandes *et al.* (2015), they found that the bactericidal potential of the ethanol extract of *H. courbaril* was better at the concentration of 127.71 μg mL^{-1}, compared to *E. coli* isolates, while in this study, the action occurred at a higher concentration (490 μg mL^{-1}). Garcia *et al.* (2011) revealed that the bactericidal action of *H. courbaril* was much higher than the other extracts tested on *S. aureus*, presenting MBC of 3.33 mg mL^{-1}.

Thus, since EOs have efficient activity against bacteria and toxicity against the non-target *Artemia salina* Leach organisms, it is important to highlight that *Artemia salina* Leach is a highly resistant microcrustacean and therefore used in models of toxicity studies of natural products, which would justify the non-lethal action of EOs in organisms.

4 CONCLUSIONS

GC/MS allowed the chemical characterization of EOs and quantified the *β-ocimene* EO as the majority of The EO and The EO of *S. cumini* isokaryophyllene.Both EOs presented atoxicity against *Artemia salina*. The antioxidant activity of The EOs was efficient. All strains tested were sensitive to bactericidal action of EOs. The results obtained are encouraged by the potential use of the OE's studied in the control and fight of pathogenic microorganisms.

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