UFSM

CIÊNCIA e NATURA

ISSN 2179-460X
OPEN ACCESS

Ci. e Nat., Santa Maria, v. 46, e73702, 2024 • https://doi.org/10.5902/2179460X73702
Submitted: 03/01/2023 • Approved: 26/06/2024 • Published: 06/09/2024

Chemistry

Chemical characterization and biological potentials of the essential oil from the peel of the fruit of *Citrus x* sp

Caracterização química e potencial biológico do óleo essencial da casca do fruto *Citrus* x sp

Amanda Torres de Queiroz[I], Ana Patrícia Matos Pereira[I], Ana Paula Muniz Serejo[I], Beatriz Jardim Rodrigues das Chagas[I], Marcelle Adriane Ataide Matos[I], Cassiano Vasques Frota Guterres[I], Victor Elias Mouchrek Filho[I], Gustavo Oliveira Everton[I]

[I] Universidade Federal do Maranhão, MA, Brazil

ABSTRACT

Considering the importance of essential oils, the study aimed to chemically characterize the peel essential oil of *Citrus x sp*, determine the phenolic compounds, verify the toxicity against *Artemia salina,* and test the anti-inflammatory, larvicides, and activities with molluscicides. The peel essential oil of *Citrus x sp* was extracted by the hydrodistillation technique. GC/MS shows that the major component of the essential oil is limonene (70.25%), observing similarity between the peel essential oil of the same species and between species of the same genus. The total phenolic was 19,25 EAT g^{-1}. The essential oil showed anti-inflammatory, larvicide, and molluscicide potential, with EC_{50} = 400.05 mg L^{-1}, LC_{50} = 20.26 µg mL^{-1} and LC_{90} = 87,50 µg mL^{-1}, respectively. It did not show toxicity against *Artemia salina*. The results indicate that the chemical compounds of the peel essential oil of *Citrus x sp*, which are mostly monoterpenes, prove their wide biological activity, and their use is encouraged.

Keywords: Essential oils; Larvicide; Molluscicide

RESUMO

Considerando a importância dos óleos essenciais, o estudo objetivou caracterizar quimicamente o óleo essencial das cascas de *Citrus x sp*, determinar os compostos fenólicos, verificar a toxicidade frente *Artemia salina* e testar as atividades anti-inflamatórias, larvicidas e moluscicidas. O óleo essencial das cascas de *Citrus x sp* foi extraído pela técnica de hidrodestilação. A CG/EM mostrou que o componente majoritário do óleo essencial é o limoneno (70,25), observando-se similaridade entre o óleo das cascas da mesma espécie e entre espécies do mesmo. O conteúdo fenólico total foi de 19,25 EAT g^{-1}. O óleo essencial apresentou potencial anti-inflamatório, larvicida e moluscicida, com CE_{50} = 400,05 mg L^{-1}, CL_{50} =

20,26 µg mL^{-1} e CL_{90} = 87,50 µg mL^{-1}, respectivamente, e não apresentou toxicidade frente *Artemia salina*. Os resultados indicam que os compostos químicos do óleo essencial das cascas de *Citrus x* sp, que são em sua maioria monoterpenos, comprovam sua ampla atividade biológica, sendo seu uso incentivado.

Palavras-chave: Óleos essenciais; Larvicida; Moluscicida

1 INTRODUCTION

In recent years, there has been a great scientific advance involving the chemical and pharmacological studies of medicinal plants, in order to obtain new compounds with therapeutic properties (Moraes et al., 2018).

Among the products obtained from medicinal plants, essential oils stand out. These consist of secondary metabolites, which are volatile, odoriferous, liquid substances with an oily appearance at room temperature, which can be extracted from leaves, flowers, fruits, stems and roots of plants. Among the substances present in essential oils, the terpenes class is mostly found, including their alcoholic and aldehyde derivatives (Aciole, 2009).

The use of essential oils, alone or in combination with other methods, plays an important role in the control of phytopathogens, contributing to the reduction in the use of pesticides and, consequently, a smaller impact on the environment, as well as in the control of disease vector organisms. as a decrease in the use of larvicides and molluscicides (Pereira et al., 2014).

Among the essential oils highlighted for such purposes are the species of the genus *Citrus*, with emphasis on *Citrus x sp*, being mainly composed of terpenes and terpenoids and widely used in the food, pharmaceutical and fine chemical industries (Ozturk et al., 2018).

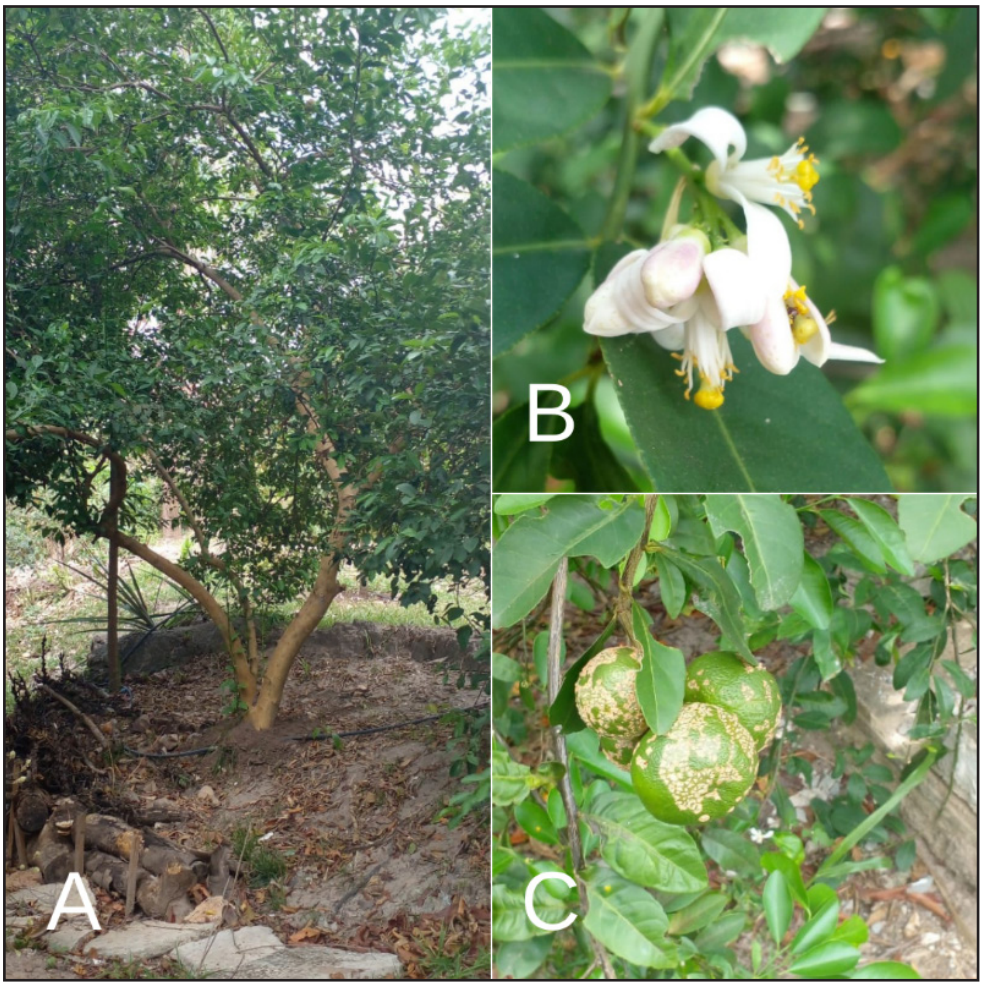
The high cultivation of this species and the continuous discarding of the fruit peels create a huge environmental problem (Nunes, 2009). Thus, the reuse of these residues in an efficient, economical and safe way for the environment becomes important. Thus, this study aimed to evaluate the chemical profile and molluscicidal activity against *Biomphalaria glabrata* and larvicide against *Aedes aegypti* of the essential oil extracted from the peel of the fruit of *Citrus x sp*.

2 METHODOLOGY

2.1 Collection of plant material

Citrus x sp peel samples were collected in May 2022 (moisture content 75.01%, chlorophyll content 0.433 g/g), in the morning in the city of São Luís. The plant material, represented in Figure 1. After collection, plant samples were transported to the Laboratory for Research and Application of Essential Oils (LOEPAV/UFMA), where they were weighed, crushed and stored for essential oil extraction.

Figure 1 – *Citrus x* sp. (a) Tree, (b) flowers, (c) fruit



Source: Authors (2022)

2.2 Essential oil extraction

To extract the essential oil, the hydrodistillation technique was used with a Clevenger glass extractor coupled to a round-bottomed flask placed in an electric blanket. 120 g of crushed *Citrus x* sp peels were used, adding if distilled water (1:8). Hydrodistillation was carried out for 3 hours at 100°C and the extracted essential oil was collected and dried by percolation with anhydrous sodium sulfate (Na_2SO_4). These operations were performed in triplicate and the samples stored under 4°C refrigeration. Subsequently, submitted to analysis.

2.3 Gas chromatography coupled to mass spectrometry

The essential oil constituents were identified by Gas Chromatography Coupled to Mass Spectrometry (GC-MS). 1.0 mg of the sample was dissolved in 1000 μL of dichloromethane (99.9% purity).

The analysis conditions were as follows: Method: Adams. M; Injected volume: 0.3 μL; Column: Capillary HP-5MS (5% diphenyl, 95% dimethyl polysiloxane) (DB-5MS equivalent or CP-Sil 8CB LB/MS), in dimensions (30m x 0.25 mm x 0.25 μm); Carrier gas: He (99.9995); 1.0 mL min^{-1} ; Injector: 280ºC, Split mode (1:10); Oven: 40 ºC (5.0 min.) to 240ºC at a rate of 4ºC min^{-1} , from 240ºC to 300ºC (7.5 min) at a rate of 8ºC.min^{-1}); tT = 60.0 min; Detector: EM; IS (70 eV); Scan mode (0.5 sec $scan^{-1}$); Mass range: 40-500 daltons (one); Line transfer: 280ºC; Filament: off 0.0 to 4.0 min; Linear quadrupole type mass spectrometer. For the identification of the compounds in the sample, the program AMDIS (Automated Mass spectral Deconvolution Mass & Identification System) was used.

2.4 Total Phenolic Content

The determination of the total phenolic compounds of the essential oil was carried out with an adaptation of the Folin-Ciocalteu method (Waterhouse, 2002). 5 mg of essential oil diluted in 1 mL of ethanol were used. To this solution, 7 mL of distilled water, 800 μL of Folin-Ciocalteu reagent and 2.0 mL of 20% sodium carbonate were

added. After two hours, the reading was performed in a UV-VIS spectrophotometer at a length of 760 nm. The standard curve was expressed in mg L^{-1} of tannic acid.

2.5 Anti-inflammatory activity

The anti-inflammatory activity was evaluated by the protein denaturation method (Padmanabhan & Jangle, 2012). The reaction mixture (5 mL) consisted of 2 mL of different concentrations of essential oil (100-500 mg L^{-1}) and 2 mL of a 10% albumin solution diluted in PBS and incubated at (37±1) °C for 15 minutes.

Denaturation was induced by keeping the reaction mixture at 60°C in a water bath for 10 minutes. After cooling, the absorbance was measured at 660 nm. Inhibition of protein denaturation was expressed as a percentage and the 50% Efficient Concentration (EC_{50}/IC_{50}) capable of inhibiting 50% of denaturation was expressed as mg L^{-1}.

2.6 Toxicity

To evaluate the lethality of *Artemia salina* Leach, a stock saline solution of each essential oil was prepared at a concentration of 10.000 mg L^{-1} and Tween 80 (active tensile). Aliquots of 5, 50 and 500 µL were transferred to test tubes and completed with previously prepared saline solution up to 5 mL, obtaining concentrations of 10, 100 and 1000 mg L^{-1}, respectively. All tests were performed in triplicate, where ten larvae in the nauplius stage were transferred to each of the test tubes.

For the blank, 5 mL of saline solution was used, for the positive control $K_2Cr_2O_7$ and for negative control 5 mL of a 4 mg L^{-1} solution of Tween 80. After 24 hours, the live larvae were counted, where the dead ones were considered as those that did not move during the observation and neither with the light shaking the flask. The criterion established by Dolabela (1997) was adopted to classify the toxicity of essential oils, being considered highly toxic when $LC_{50} \leq 80$ mg L^{-1}, moderately toxic when 80 mg $L^{-1} \leq LC_{50} \geq 250$ mg L^{-1} and slightly toxic or non-toxic when $LC_{50} \geq 250$ mg L^{-1}. Statistical analysis of the data is carried out according to the Reed & Muench method (1938).

2.7 Larvicidal activity

The eggs were collected at the Federal University of Maranhão, Campus Bacanga in São Luís/MA, through traps called ovitraps. Traps were inspected weekly to replace straws and collect eggs. Initially, *Aedes aegypti eggs* were placed to hatch at room temperature in a circular glass aquarium containing mineral water. The species followed the methodology proposed by Forattini (1962). The obtained larvae were fed with cat food according to methodology Silva et al. (1995) until reaching the third and fourth stage, age at which the experiments were performed.

The larvicidal activity tests were carried out according to the adapted methodology proposed by Silva (2006). Initially, a mother solution of 100 mg L^{-1} of each essential oil was prepared and diluted in a 2% DMSO solution. Dilutions were prepared from this solution in concentrations of 10-90 mg L^{-1}.

At each concentration of essential oil, 10 larvae were added at a rate of 1 ml per larva. All tests were performed in triplicate and a solution made up of 2% DMSO was used as a negative control and a 100 ppm temephos solution was used as a positive control, equivalent to the concentration used by the National Health Foundation (FUNASA) for larvicidal control of the vector.

After 24 hours, the living and dead were found, and the larvae that did not react to the touch after 24 hours of the beginning. The beginning of the experiment was considered dead. Statistical analysis of the data was performed according to the Probit method (Finney, 1952).

2.8 Molluscicide activity

For the evaluation of the molluscicide activity, the technique recommended by the World Health Organization (1983) was applied, where two tests are performed. In the first, called pilot test, a solution of the essential oil under study was prepared in a volume of 500 mL at a concentration of 100 mg L^{-1} in Tween 80 2% (surfactant), where 10 adult snails, negative for *Schistosoma mansoni*, obtaining in the end a ratio of 50 mL/snail and fed with hydroponic lettuce.

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

In the second test, the Lethal Concentration (LC_{50}) was evaluated, where essential oil solutions were prepared in a volume of 500 mL at concentrations 100, 80, 60, 40, 20, 10 and 5 mg L^{-1} in 2% Tween 80 (active voltage), using the same methodology as the pilot test. Positive, negative and blank controls were also performed. Mortality rates were obtained by averaging the number of individuals killed as a function of the logarithm of the tested dose.

Statistical analysis of the data for the CL_{50} was performed according to the Reed&Muench method (1938) and the confidence interval using the Pizzi method (1950).

3 RESULTS AND DISCUSSION

3.1 Chemical constituents

Ten (10) chemical constituents were identified using the GC/MS technique in the essential oil, namely: limonene (70.25%, monoterpene), β-pinene (21.12%, monoterpene), α–pinene (3, 01%, monoterpene), β-bisabolene (2.10%, sesquiterpene), linalool (1.00%, monoterpene), terpinen-4ol (0.98%, monoterpene), β-myrcene (0.81%, monoterpene), pinocarvone (0.41%, monoterpene), caryophyllene oxide (0.22%, terpenoid) and carvone (0.10%).

Among the terpene compounds, the most frequent in essential oils are monoterpenes and sesquiterpenes (Ribeiro, 2016), with limonene being the main component of essential oils from the peel of fruits of the genus *Citrus*, between 30 and 70% of the composition (Amorim et al., 2016), corroborating the result obtained in the essential oil of the peels of *Citrus x* sp.

Similar results were observed by Sousa (2019) when identifying 20 chemical components, corroborating the majority compound observed in this study, which

can be justified by the considerable dependence on secondary metabolites and the interactions of biochemical, physiological, ecological and evolutionary processes, which results in a variation in the production of secondary metabolites often affected by environmental conditions.

Essential oils rich in monoterpenes, as observed in this study, are recognized as food preservatives, considered natural antioxidants and reported as anticancer (Singh et al., 2010). The literature shows that limonene, the main chemical component of the essential oil, has selective antimicrobial, antifungal, and antioxidant activity, in addition to having a promoting effect on the passage of a drug for skin cancer through the skin barrier (Vuuren&Viljoen, 2007; Jing et al., 2014; Thomas&Bessière, 1989; Bertolini, 2009).

3.2 Total Phenolic Content

The result of the quantification of the total phenolic content in the essential oil of the peels of *Citrus x* sp is shown in Table 1. The total phenolic content (TLC) was expressed as tannic acid equivalents (mg EAT g^{-1} of plant material) the equation of The line obtained was y = 0.05857x + 0.06000 (R^2 =0.9998), where y represents the absorbance x the equivalent concentration of tannic acid.

Table 1 – Total phenolic content of *Citrus x* sp

Essential oil	TPC (mg EAT g^{-1})	Equation	R^2
Citrus x sp	1.925 EAT g^{-1}	y = 0.05857x + 0.06000	0.9998

Source: Queiroz (2023)

Siahposh&Javedani (2012) showed that the total phenolic content of the extract of *Citrus delicacy peels* was 92.08 ± 2.50 mg. Khan et al. (2012) presented for the essential oils of the bark of *Citrus sinensis* Vars. Jaffa and *Citrus reticulata* Var. Kinnow the values of 6.18±2.02 and 5.20±0.02, respectively.

Using the method of ethanolic extraction of phenolic compounds from fresh peels, Li et al. (2006) found, for *Citrus. x paradisi* 161.60±17.36 mg GAE, *Citrus. reticulata* cv. Ellendale 121.14 ± 11.89 mg GAE, *Citrus limon* cv. Yenben 118.95 ± 9.35 mg GAE, *Citrus sinensis* cv. Navel 73.59 ± 5.43 mg GAE and *Citrus limon* cv. Meyer 59.77 ± 4.31 mg GAE. Furlong et al. (2003), analyzing the ethanolic extract of *Citrus sinensis* Osbeck, obtained the value of 697.70 µg phenol g^{-1}, which shows a wide range of values and methods for quantification of total phenolic compounds.

The total phenolic content of the leaves of the essential oil of *Citrus x* sp observed is important for the antioxidant study of the species, which is scarce, since these substances are synthesized to act in the absorption of radiation in the epidermal layers of tissues, regulating the antioxidant system in the tissues. cells (Gobbo-Neto, 2007). They are also responsible for the therapeutic action of plants, which have three main classes of substances: terpenes, phenolic and nitrogenous compounds (Borges&Amorim, 2020).

3.3 Anti-inflammatory

The results obtained for the anti-inflammatory action regarding the analyzed essential oil are shown in Table 2.

Table 2 – Efficient Concentration 50% (EC_{50}/IC_{50}) for anti-inflammatory action of *Citrus x* sp

Essential oil	EC_{50}/IC_{50} (mg L^{-1})	Equation	R^2
Citrus x sp	400.05	y = 195.56x – 458.87	0.9997

Source: Queiroz (2023).

According to Table 2, the essential oil was classified as active, according to the criteria of Padmanabhan & Jangle (2012). The observed anti-inflammatory activity is described for the genus *Citrus*, being reported in several studies (Huang&Ho, 2010; Moraes, 2012; Amorim et al., 2016).

The anti-inflammatory effect of the essential oil from the peels of *Citrus x sp*

can be explained by the presence of terpenes, since its main constituent is limonene, one of the main constituents in the aforementioned essential oils of the genus *Citrus,* reinforcing the hypothesis of its importance in anti-inflammatory action. This anti-inflammatory potential for the limonene constituent was described in Hirota et al. (2010), Moraes (2012), Krummer (2011) and Yu (2017).

Still, studies with other species of the genus show their anti-inflammatory potential, such as *Citrus myrtifolia* Raf., *Citrus aurantium* L., *Citrus aurantifolia, Citrus x limon and Citrus latifolia* Tanaka are examples of species that have moderate anti-inflammatory activity discharge, with different biological responses (Krummer, 2011; Hamdan et al., 2013; Plastina et al.; Dosoky & Setzer, 2018;).

The anti-inflammatory potential of essential oils, as observed in this study, is attributed to their ability to scavenge free radicals, since the inflammatory response normally involves oxidative stress, and to their interactions with signaling cascades involving cytokines and regulatory transcription factors, and in the expression of pro-inflammatory genes (Miguel, 2010; Boscardin, 2012).

The potential observed for the studied essential oil could be used for the treatment of inflammations, which are physiological responses to a variety of agents, including infectious microorganisms, toxic substances and physical injuries. There are several diseases associated with inflammatory processes, such as skin inflammation, autoimmune diseases, Alzheimer's and cancer (Pérez et al., 2011). Thus, the essential oil obtained proves to be a bioproduct with broad biotechnological potential with a broad spectrum of application.

3.4. Toxicity

Table 3 shows the toxicity assessment of the essential oil analyzed against *Artemia salina* Leach.

According to Table 3, in the toxicity test, LC_{50} above 1000 mg L^{-1} was proven for the action of the essential oil against *Artemia salina* Leach, being classified as non-

toxic by the criteria of Dolabela (1997), which says that the product is highly toxic when $LC_{50} \leq 80$ µg mL^{-1}, moderately toxic for µg $mL^{-1} \leq LC_{50} \geq 250$ µg mL^{-1} and slightly toxic or non-toxic when $LC_{50} \geq 250$ µg mL^{-1}, encouraging the potential application of the essential oil.

Table 3 – Assessment of essential oil toxicity against the non-target organism *Artemia salina* Leach

LC_{50}	**>1000 mg L^{-1} (µg mL^{-1})**
Positive control (Potassium dichromate)	All inactive larvae
White (saline solution)	All active larvae
Negative control (saline+Tween80)	All active larvae

Source: Queiroz (2023)

The result obtained corroborates that found by Sousa (2019), who classified the essential oil from the peels of *Citrus x* sp as non-toxic. The applicability of essential oil from other species of the genus is found in the literature, such as essential oils from the epicarp and aerial parts of *Citrus x limon* (L.) Osbeck, which were classified as moderately toxic, and the essential oil from the bark of *Citrus sinensis* that have a LC_{50} of 511.6 mg L^{-1}, thus being classified as non-toxic (Carvalho, 2018; Oliveira, 2021).

The positive activity found in vitro in tests with *Artemia salina* Leach works as a previous evaluation of substances with pharmacological potential to be evaluated in more specific bioassays, such as antitumor, antifungal, insecticide and trypanocidal potential (Harada, 2009; Gomes et al., 2018). Positive results finally reinforce the biological properties reported in the previous topics, again reinforcing the biotechnological potential found.

3.5. Larvicidal activity

Table 4 shows the LC_{50} of the essential oil extracted from the bark of *Citrus x* sp against *Aedes aegypti* larvae.

As observed in Table 4, there was mortality of larvae in 100% up to the concentration of 40 mg L^{-1}. At concentrations of 30-15 mg L^{-1} there was a gradual decrease in the mortality rate, still appearing at 30% at the reported lower limit. Mortality reduced considerably from the decrease in concentrations below 15 mg L^{-1}.

Table 4 – Lethal Concentration 50% for essential oil action against *Aedes aegypti* larvae

Concentration (mg L^{-1})	**Mortality (%)**	**LC_{50} (mg L^{-1})**	**σ**	**χ^2**	**R^2**
100.00	100.0				
80.00	100.0				
60.00	100.0				
40.00	100.0	20.26			
30.00	80.0	(16.26-25.26)	0.232	0.781	0.965
25.00	60.0				
15.00	30.0				
5.00	0.0				
0.00	0.0				
Positive Control				All active larvae	
Negative Control				All inactive larvae	
White				All inactive larvae	

Source: Queiroz (2022)

The LC_{50} presented in Table 4, showing the larvicidal potential of the essential oil of the peels of *Citrus x* sp, allowed classifying it as very active, according to the criteria of Cheng et al. (2003), which establishes that very active essential oils have LC_{50} lower than 50 mg L^{-1}. The related studies are still little published in technical-scientific journals, reinforcing the importance of this study.

The result is in line with Sousa (2019), where LC_{50} = 20.26 mg L^{-1} was also found. Furthermore, the larvicidal potential of the genus *Citrus* is strongly described in the literature, where studies demonstrate the Lethal Concentration 50% for testing with essential oils such as *Citrus limon* L. (LC_{50} = 14.99 µg mL^{-1}), *Citrus sinensis* L. Osbeck (LC_{50}= 99.10 µg mL^{-1}), *Citrus aurantifolia* (LC_{50}=69.71 µg

mL^{-1}), *Citrus reticulata* Blanco (LC_{50} = 58.35 µg mL^{-1}) (Oliveira, 2013; Assunção, 2013; Sousa, 2019; Oliveira et al., 2021).

Studies show that terpene constituents, alcohols and aldehydes of essential oils are mainly responsible for larvicidal and insecticidal activity (Mendes, 2012). Limonene is mentioned by Govindarajan et al. (2011) as an effective larvicide against *Aedes aegypti* larvae.The presence of lipophilic groups, such as β-pinene, results in increased larvicidal potential, and this component, along with α-pinene and other terpenes, is cytotoxic, lipophilic, bactericidal, fungicidal, insecticide, anticarcinogenic, pesticide, antioxidant and sedative (Santos et al., 2011; Oliveira et al., 2016).

3.6. Molluscicide activity

Table 5 shows the LC_{50} and LC_{90} of the essential oil extracted from the peels of *Citrus x* sp against the snails *Biomphalaria glabrata*.

Table 5 – Lethal Concentration 50% and 90% of essential oil against *B. glabrata snails*

Concentration (mg L^{-1})	**% Mortality**	**LC_{50} mg L^{-1}**	**LC_{90} mg L^{-1}**	**δ**	**χ^2**	**R ^{2}**
200.00	100					
175.00	100					
100.00	100					
90.00	100					
80.00	81.4	39.27 (29.76-51.82)	87.50 (77.99-100.05)	0.414	0.986	0.967
70.00	73.6					
50.00	61.2					
40.00	41.5					
20.00	20					
10.00	10					
Positive Control				All inactive snails		
Negative Control				All active snails		
White				All active snails		

Source: Queiroz (2022)

LC_{90} was observed at 87.50 mg L^{-1}, and according to the criteria of WHO (1983), a plant with molluscicide properties is considered active if it causes 90% of mortality in concentrations of up to 100 mg L^{-1}, so the essential oil tested against *B. glabrata snails* proved to be active for the test used.

Even more significant results were obtained by Fernandes (2011), who studied the molluscicide activity against *B. glabrata* of the essential oil of *Citrus limon* L and obtained a LC_{50} of 13.18 mg L^{-1} when compared to the CL_{50} obtained in this study, which was 39.27 mg L^{-1}. Comparing the LC_{90} = 168.75 µg mL^{-1} obtained by Leite Júnior (2018) in *Citrus sinensis* (L.) Osbeck, the result obtained in this study is satisfactory and within the recommended by WHO (1983).

This is the first time that the essential oil from the bark of *Citrus* x sp is subjected to tests of molluscicidal activity against adult snails of the species *Biomphalaria glabrata*, however the biological activity of essential oils is directly related to their chemical composition, and this relationship is often times suggests that the biological activity of an essential oil can be attributed both to its major components and to components in lower concentration, in addition to the possibility that they act in synergy, contributing to the total toxicity of the essential oil (Jorge, 2017).

Still, in Martins et al. (2016), the *in vitro schistosomicidal activity* of *C. limonia* and *C. reticulata oils* against *S. mansoni* showed that the schistosomicidal effect was not entirely attributed to limonene, pointing to a possible synergy between the chemical components of the oils. The use of essential oil from the bark of *Citrus x* sp is encouraged, as it has been shown to be active against *Biomphalaria glabrata* in amounts recommended by WHO (1983).

3 CONCLUSIONS

This study showed that the chemical profile of the essential oil from the peel of *Citrus x* sp has components of broad biological potential. The toxicity test against *Artemia salina* demonstrated that the EO is not toxic to non-target organisms. Still, regarding

the larvicidal and molluscicide activities, it was qualified as efficient, presenting itself with the potential to control populations of *Aedes aegypti* and *Biomphalaria glabrata*. With this, the wide use of the essential oil of the peels of *Citrus x* sp is encouraged.

REFERENCES

Aciole, S. D. G. (2009). Avaliação da actividade insecticida dos óleos essenciais nas plantas amazônicas Annonaceae, Boraginaceae e de Mata Atlântica Myrtaceae como alternativa de controle às larvas de Aedes aegypti (Linnaeus, 1762) (Diptera: Culicidae). 2009. [Doctoral dissertation]- Universidade de Lisboa, Potugal.

Amorim, J. L. et al. (2016). Anti-inflammatory properties and chemical characterization of the essential oils of four citrus species. *PloS one*, 11(4), e0153643. doi:10.1371/journal.pone.0153643.

Assunção, G. V. D. (2013). Caracterização química e avaliação da atividade larvicida frente ao Aedes aegypti do óleo essencial da espécie Citrus sinensis l. Osbeck (laranja doce). 2013 [Dissertação Mestrado em Quimica] - Universidade Federal do Maranhão, Brasil.

Bertolini, W. L. H. M. (2009). A influência do D-limoneno como promotor de absorção de ácido 5-aminolevulínico para Terapia Fotodinâmica do câncer de pele: avaliação in vitro e in vivo da permeação e retenção cutâneas. 2009. [Doctoral dissertation] - Universidade de São Paulo.

Borges, L. P., & Amorim, V. A. (2020). Secondary plant metabolites; *Revista Agrotecnologia*, 11(1), 54-67. doi: 10.5555/20203146233.

Boscardin, P. M. D. (2012). Avaliação anti-inflamatória e citotóxica do óleo essencial de Eucalyptus Benthamii Maiden et Cambage.

Campos, J. M. et al. (2018). Arboviroses de importância epidemiológica no Brasil. *Revista de Ciências da Saúde Básica e Aplicada*, 1(1).

Carvalho, A. C. M. (2018). Controle da esquistossomose: atividade moluscicida e cercaricida do óleo essencial de Citrus x limon (L.) Osbeck.2018. [Dissertação Mestrado em Saúde e Ambiente] - Universidade Federal do Maranhão.

Cheng, S. S. et al. (2003). Bioactivity of selected plant essential oils against the yellow fever mosquito Aedes aegypti larvae. *Bioresource Technology*, 89(1), 99-102.doi: 10.1016/S0960-8524(03)00008-7.

Dolabela, M. F. (1997). Triagem in vitro para atividade antitumoral e anti-T. cruzi de extratos vegetais, produtos naturais e substâncias sintéticas. Belo Horizonte.1997. [Dissertação Mestrado] –Universidade Federal de Minas Gerais.

Dosoky, N. S., & Setzer, W. N. (2018). Biological activities and safety of Citrus spp. essential oils. International *Journal of Molecular Sciences*, 19(7), 1966.doi: 10.3390/ijms19071966.

Fernandes, R. P. (2011). Caracterização química, avaliação da toxicidade e atividade moluscicida dos óleos essenciais da folha de Pimenta dióica Lindl, casca de Citrus limon Linneo e Rizoma de Zingiber officinale Roscoe.2011. [Tese Doutorado em Química] - Universidade Federal da Paraíba.

Finney, D. J. (1952). Probit analysis: a statistical treatment of the sigmoid response curve. Cambridge University Press, Cambridge.

Forattini, O. P. (1962). Entomologia médica, vol. 1. Universidade de São Paulo, São Paulo, 185-302.

Furlong, E. B. et al. (2003). Avaliação do potencial de compostos fenólicos em tecidos vegetais. VETOR-Revista de Ciências Exatas e Engenharias, 13, 105-114. https://periodicos.furg.br/vetor/article/view/433

Garcez, W. S. et al. (2013). Substâncias de origem vegetal com atividade larvicida contra Aedes aegypti. *Revista Virtual de Química*, 5(3), 363-393. doi: 10.5935/1984-6835.20130034.

Gobbo-Neto, L., & Lopes, N. P. (2007). Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. *Química nova*, 30(2), 374-381. doi: 10.1590/S0100-40422007000200026.

Gomes, P. R. B. et al. (2018). Caracterização química e citotoxicidade do óleo essencial do cravo-da-índia (Syzygium aromaticum). *Revista Colombiana de Ciencias Químico-Farmacéuticas*, 47(1), 37-52. doi: 10.15446/rcciquifa.v47n1.70657.

Govindarajan, M. et al. (2011). Chemical composition and larvicidal activity of essential oil from Mentha spicata (Linn.) against three mosquito species. *Parasitology Research*, 110(5), 2023–2032. doi: 10.1007/s00436-011-2731-7.

Hamdan, D. et al. (2013). Chemical composition of the essential oils of variegated pink-fleshed lemon (Citrus x limon L. Burm. f.) and their anti-inflammatory and antimicrobial activities. *Zeitschrift für Naturforschung C*, 68(7-8), 275-284. doi: 10.1515/znc-2013-7-804.

Harada, T. N. (2009). Correlação entre os ensaios de citotoxicidade em Artemia salina Leach e atividade antineoplásica sobre linhagens de células tumorais para algumas classes de produtos naturais. 2009. [Dissertação Mestrado em Saúde e Desenvolvimento] – Universidade Federal do Mato Grosso do Sul.

Hirota, R. et al. (2010). Anti□inflammatory Effects of limonene from yuzu (Citrus junos Tanaka) essential oil on eosinophils. *Journal of food science*, 75(3), H87-H92. Doi:10.1111/j.1750-3841.2010.01541.x.

Huang, Y. S., & Ho, S. C. (2010). Polymethoxy flavones are responsible for the anti-inflammatory activity of citrus fruit peel. *Food Chemistry*, 119(3), 868-873. doi:10.1016/j.foodchem.2009.09.092.

Jing, L. et al. (2014). Antifungal activity of citrus essential oils. *Journal of agricultural and food chemistry*, 62(14), 3011-3033. doi:10.1021/jf5006148.

Jorge, N. A. (2017). Estudo químico e avaliação da atividade moluscicida do óleo essencial de Citrus limon L.(limão) frente ao caramujo transmissor da esquistossomose (Biomphalaria glabrata). 2017. [Dissertação em bacharel em Química Industrial] - Universidade Federal do Maranhão.

Khan, M. M., Iqbal, M., Hanif, M. A., Mahmood, M. S., Naqvi, S. A., Shahid, M., & Jaskani, M. J. (2012). Antioxidant and antipathogenic activities of citrus peel oils. *Journal of Essential Oil Bearing Plants*, 15(6), 972-979. doi:10.1080/0972060X.2012.10662601.

Kummer, R. (2011). Avaliação da atividade anti-inflamatória do óleo essencial de Citrus latifolia Tanaka e limoneno em modelos experimentaiis em camundongos. 2011. [Dissertação Mestrado em Ciências Farmacêuticas] - Universidade Estadual de Maringá.

Leite Júnior, J. D. D. D. (2018). Avaliação da atividade moluscicida do óleo essencial de Citrus sinensis (L.) Osbeck frente aos caramujos transmissores da esquistossomose. 2018. [Dissertação em Saúde e Ambiente] - Universidade Federal do Maranhão.

Li, B. B., Smith, B., & Hossain, M. M. (2006). Extraction of phenolics from citrus peels: I. Solvent extraction method. *Separation and Purification Technology*, 48(2), 182-188. doi: 10.1016/j.seppur.2005.07.005.

Lopes, N., Nozawa, C., & Linhares, R. E. C. (2014). Características gerais e epidemiologia dos arbovírus emergentes no Brasil. *Revista Pan-Amazônica de Saúde*, 5(3), 10-10. doi: 10.5123/S2176-62232014000300007.

Martins, M. H. et al. (2017). Schistosomicidal effects of the essential oils of Citrus limonia and Citrus reticulata against Schistosoma mansoni. *Chemistry & Biodiversity*, 14(1), e1600194. doi:10.1002/cbdv.201600194.

Mendes, L. S. D. S. (2012). Estudo químico e atividade larvicida frente ao Aedes aegypti do óleo essencial das folhas de Cinnamomum zeylanicum Breyn (canela). 2012.[Dissertação Mestrado em Química] - Universidade Federal do Maranhão.

Miguel, M. G. (2010). Antioxidant and anti-inflammatory activities of essential oils: a short review. *Molecules,* 15(12), 9252-9287.doi: 10.3390/molecules15129252.

Moraes, R. P., Correa, I. C., & Vanin, A. B. (2018). toxicidade dos óleos essenciais de endro (Anethum graveolens) e nim (Azadirachta indica A. Juss) através do ensaio de bioletalidade utilizando Artemia salina. Seminário de Iniciação Científica e Seminário Integrado de Ensino, Pesquisa e Extensão.

Moraes, T. D. M. (2012). Avaliação do óleo essencial de Citrus aurantium e do seu constituinte majoritário, limoneno, como adjuvantes no tratamento da artrite reumatóide em ratos com úlceras gástricas. 2012.[Tese doutorado em Ciências Biológicas] - Instituto de Biociências, Universidade Estadual Paulista.

Nunes, J. T. (2009). Aproveitamento integral dos alimentos: qualidade nutricional e aceitabilidade das preparações. 2009.[Monografia Especialização em Qualidade de Alimentos] -Universidade de Brasília.

Oliveira, A. C. S. D. et al. (2021). Composição química, atividades larvicida e leishmanicida in vitro do óleo essencial da casca do fruto de Citrus reticulata Blanco. *Brazilian Journal of Biology*, 83, e247539. doi: 10.1590/1519-6984.247539.

Oliveira, J. D. et al. (2016). Rendimento, composição química e atividades antimicrobiana e antioxidante do óleo essencial de folhas de Campomanesia adamantium submetidas a diferentes métodos de secagem. *Revista Brasileira de Plantas Medicinais*, 18, 502-510. doi:10.1590/1983-084X/15_206.

Oliveira, M. B. (2013). Extração, caracterização e avaliação da atividade larvicida do óleo essencial do Citrus limon Linneo (limão) frente ao mosquito Aedes aegypti. 2013. [Dissertação Mestrado em Química] - Universidade Federal do Maranhão.

Organización Mundial de la Salud. *Esquistosomiasis*. (2021). Retrieved from: https://www.who.int/es/news-room/fact-sheets/detail/schistosomiasis.

World Health Organization. (1983). Report of the scientific working group on plant molluscicides. *Bull World Health Organ,* 12, 1-11.

Ozturk, B., Esteban, J., & Gonzalez-Miquel, M. (2018). Deterpenation of citrus essential oils using glycerol-based deep eutectic solvents. *Journal of Chemical & Engineering Data*, 63(7), 2384-2393. doi: 10.1021/acs.jced.7b00944.

Padmanabhan, P., & Jangle, S. N. (2012). Evaluation of in-vitro anti-inflammatory activity of herbal preparation, a combination of four medicinal plants. *International journal of basic and applied medical sciences*, 2(1), 109-116. http://www.cibtech.org/jms.htm.

Pereira, Á. I. S. et al. (2014). Atividade antimicrobiana no combate às larvas do mosquito Aedes aegypti: homogeneização dos óleos essenciais do linalol e eugenol. *Educación química*, 25(4), 446-449. doi:10.1016/S0187-893X(14)70065-5.

Pérez G, S. et al. (2011). Anti-inflammatory activity of some essential oils. *Journal of Essential Oil Research*, 23(5), 38-44. doi:10.1080/10412905.2011.9700480.

Pizzi, M. (1950). Sampling variation of the fifty per cent end-point, determined by the ReedMuench (Behrens) method. *Human biology*, 22(3), 151-190.

Plastina, P. et al. (2018). In vitro anti-inflammatory and radical scavenging properties of chinotto (citrus myrtifolia raf.) essential oils. *Nutrients*, 10(6), 783. doi:10.3390/nu10060783.

Reed, L. J., & Muench, H. (1938). A simple method of estimating fifty per cent endpoints. *American journal of epidemiology*, 27(3), 493-497.

Ribeiro, E. C. G. (2016). Atividade moluscicida de óleos essenciais de plantas aromáticas da região Amazônica maranhense. 2016. [Dissertação Mestrado em Saúde e Ambiente] - Universidade Federal do Maranhão.

Santos, S. R. et al. (2011). Structure–activity relationships of larvicidal monoterpenes and derivatives against Aedes aegypti Linn. *Chemosphere*, 84(1), 150-153. doi:10.1016/j.chemosphere.2011.02.018.

Siahpoosh, A., & Javedani, F. (2012). Antioxidative capacity of Iranian Citrus deliciosa peels. *Free Radicals and Antioxidants*, 2(2), 62-67. Doi:10.5530/ax.2012.2.2.11.

Silva, H. et al. (1995). Idade fisiológica dos ovos de Aedes (stegomyia) aegypti (Linnaeus, 1762) (diptera, culicidae). *Rev. Patol. Trop*, 269-73.

Silva, W. J. D. (2006). Atividade larvicida do óleo essencial de plantas existentes no estado de Sergipe contra Aedes aegypti Linn. 2006. [Dissertação Mestrado em Desenvolvimento e Meio Ambiente] - Universidade Federal de Sergipe.

Singh, P. et al. (2010). Chemical profile, antifungal, antiaflatoxigenic and antioxidant activity of Citrus maxima Burm. and Citrus sinensis (L.) Osbeck essential oils and their cyclic monoterpene, DL-limonene. *Food and Chemical Toxicology*. 48(6), 1734-1740. doi:10.1016/j.fct.2010.04.001.

Sousa, D. A. D. (2019). Avaliação da atividade larvicida de óleos essenciais de espécies de citrus frente às larvas de Aedes aegypti.2019. [Dissertação em Saúde e Ambiente] - Universidade Federal do Maranhão.

Thomas, A. F., & Bessiere, Y. (1989). Limonene. *Natural Product Reports*, 6(3), 291-309. doi: 10.1039/NP9890600291.

Vuuren, S. V., & Viljoen, A. M. (2007). Antimicrobial activity of limonene enantiomers and 1, 8 cineole alone and in combination. *Flavour and fragrance journal*. 22(6), 540-544. doi: 10.1002/ffj.1843.

Waterhouse, A. L. (2002). Determination of total phenolics. *Current Protocols in Food Analytical Chemistry*, 6(1), I1-1.

World Health Organization. Field Use of Molluscicides In Schistosomiasis Control Programmes: An Operational Manual For Programme Managers. (2017). Retrieved from: https://www. apps.who.int/iris/bitstream/handle/10665/254641/9789241511995-eng.pdf.

World Health Organization. Guidelines for laboratory and field testing of molluscicides for control of schistosomiasis. (2019). Retrieved from https://apps.who.int/iris/bitstream/handle/10665/311588/9789241515405-eng.pdf?ua=1.

Yu, L., Yan, J., & Sun, Z. (2017). D-limonene exhibits anti-inflammatory and antioxidant properties in an ulcerative colitis rat model via regulation of iNOS, COX-2, PGE2 and ERK signaling pathways. *Molecular medicine reports*, 15(4), 2339-2346. doi: 10.3892/mmr.2017.6241

Authorship contributions

1 – Amanda Torres de Queiroz

Universidade Federal do Maranhão - Graduated in Biological Sciences
https://orcid.org/0000-0003-4459-7649 - amandaqueirz@outlook.com
Contribution: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review editing

2 – Ana Patrícia Matos Pereira

Universidade Federal do Maranhão – Master in Health and Enviroment
https://orcid.org/0000-0003-4478-4209 - ap.matos11@hotmail.com
Contribution: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review editing

3 – Ana Paula Muniz Serejo

Universidade Federal do Maranhão - PhD in Biothecnology
https://orcid.org/0000-0002-4376-4364 - apsmuniz1@gmail.com
Contribution: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review editing

4 – Beatriz Jardim Rodrigues das Chagas

Universidade Federal do Maranhão - Graduating in Industrial Chemistry
https://orcid.org/0000-0002-8940-0064 - jardimbeattriz@gmail.com
Contribution: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review editing

5 – Marcelle Adriane Ataide Matos

Universidade Federal do Maranhão - Graduating in chemistry
https://orcid.org/0000-0001-5338-8123 - marcelle.mattooss@gmail.com
Contribution: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review editing

6 – Cassiano Vasques Frota Guterres

Universidade Federal do Maranhão - Graduating in chemistry
https://orcid.org/0000-0003-2725-9429 - cassianovasques447@gmail.com
Contribution: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review editing

7 – Victor Elias Mouchrek Filho

Universidade Federal do Maranhão - PhD in chemistry
https://orcid.org/0000-0003-2855-7292 - gustavo.oliveira@discente.ufma.br
Contribution: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review editing

8 – Gustavo Oliveira Everton

Universidade Federal do Maranhão - Chemistry PhD student
https://orcid.org/0000-0002-0457-914X - gustavooliveiraeverton@gmail.com Contribution:
Contribution: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review editing

How to quote this article

Queiroz, A. T. de, Pereira, A. P. M., Serejo, A. P. M., Chagas, B. J. R. das, Matos, M. A. A., Guterres, C. V. F., Mouchrek Filho, V. E., & Everton, G. O (2024). Chemical characterization and biological potentials of the essential oil from the peel of the fruit of Citrus x sp. *Ciência e Natura,* 46, e73702. https://doi.org/10.5902/2179460X73702.