Research, Society and Development, v. 11, n. 15, e432111536785, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i15.36785

Larvicidal effect of the essential oil of *Curcuma xanthorrhiza* (ginger java) for *Aedes aegypti*

Efeito larvicida do óleo essencial de *Curcuma xanthorrhiza* (gengibre java) para *Aedes aegypti*

Efecto larvicida del aceite esencial de *Curcuma xanthorrhiza* (jengibre javo) para *Aedes aegypti*

Received: 10/20/2022 | Revised: 11/08/2022 | Accepted: 11/16/2022 | Published: 11/22/2022

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Abstract
The larvicidal effect of the essential oil nanoemulsions of *Curcuma xanthorrhiza* (known as ginger java) in *Aedes aegypti* species. For this, they were collected as plants of *C. xanthorrhiza* Luís, MA and, later, separated as leaves from their respective branches. The essential oil was obtained by means of hydrodistillation by dragging a steam at a temperature of 100ºC for 3 hours. The nanoemulsions were prepared using the phase inversion method. The chemical constituents were quantified and identified by Gas Chromatography Coupled with Mass Spectrometry (GC/MS). *Aedes aegypti* eggs were transmitted through ovit traps. As *Aedes aegypti* larvae, they were hatched in mineral water,

identified and fed until the 4th instar. For the activity of the activity. living and dead count. A Probit statistical analysis was performed to quantify the Lethal Concentration 50% (LC50). The GC/MS quantified the major components in the essential oil, namely germacrone (25.46%), xanthorrizole (17.52%) and eucalyptol (11.92%). The larvicidal activity of the essential oil nanoemulsion for *Aedes aegypti* quantified an LC50 of 25.94 mg. L^{-1}, being defined by the evaluation criterion as active in the control and combat of *A. aegypti*. The essential nanoemulsion of *C. xanthorrhiza* has efficient larvicidal activity, so there is a need for further studies of its form of operation and safety for the combat and control of *Aedes aegypti* larvae.
Keywords: *Aedes*; Nanoemulsion; Essential oil.

Resumo
Avaliou-se o efeito larvicida das nanoemulsões do óleo essencial de *Curcuma xanthorrhiza* (conhecido como gengibre java) em espécie de *Aedes aegypt*i. Para isto, foram coletadas as plantas de *C. xanthorrhiza* em São Luís, MA e, posteriormente, separadas as folhas de seus respectivos galhos. O óleo essencial foi obtido por meio de hidrodestilação por arraste a vapor sob temperatura de 100ºC durante 3 horas. As nanoemulsões foram preparadas através do método de inversão de fases. Os constituintes químicos foram quantificados e identificados por Cromatografia Gasosa Acoplada a Espectrometria de Massas (CG/EM). Os ovos *Aedes aegypti* foram coletados através de armadilhas ovitrampas. As larvas *Aedes aegypti* foram eclodidas em água mineral, identificadas e alimentadas até o 4° instar. Para avaliação da atividade larvicida adotou-se os padrões regulamentados pela Organização Mundial da Saúde, sendo aplicada a metodologia de letalidade, onde submeteram grupos de 10 larvas a concentrações da nanoemulsão (10-90 mg. L^{-1}) a uma exposição de 24h para contagem de vivas e mortas. A análise estatística Probit foi adotada para quantificação da Concentração Letal 50% (CL_{50}). A CG/EM quantificou os compostos majoritários no óleo essencial, sendo eles germacrona (25,46%), xantorrizol (17,52%) e eucaliptol (11,92%). A atividade larvicida da nanoemulsão do óleo essencial para *Aedes aegypti* quantificou uma CL_{50} 25,94 mg. L^{-1}, sendo classificado pelo critério de avaliação como ativo no controle e combate de *A. aegypti*. A nanoemulsão do óleo essencial de *C. xanthorrhiza* apresenta atividade larvicida eficiente, dessa forma há necessidade de mais estudos de sua eficácia e segurança para o combate e controle das larvas de *Aedes aegypti*.
Palavras-chave: *Aedes*; Nanoemulsão; Óleo essencial.

Resumen
El efecto larvicida de las nanoemulsiones de aceite esencial de *Curcuma xanthorrhiza* (conocida como jengibre java) en especies de *Aedes aegypti*. Para ello, se colectaron como plantas de *C. xanthorrhiza* Luís, MA y, posteriormente, se separaron como hojas de sus respectivas ramas. El aceite esencial se obtuvo mediante hidrodestilación arrastrando un vapor a una temperatura de 100ºC durante 3 horas. Las nanoemulsiones se prepararon mediante el método de inversión de fase. Los componentes químicos se cuantificaron e identificaron mediante cromatografía de gases acoplada a espectrometría de masas (GC/MS). Los huevos de *Aedes aegypti* se transmitieron a través de trampas de ovit. Como larvas de *Aedes aegypti*, se incubaron en agua mineral, se identificaron y se alimentaron hasta el 4° estadio. Por la actividad de la actividad. recuento de vivos y muertos. Se realizó un análisis estadístico Probit para cuantificar la Concentración Letal 50% (LC50). La GC/MS cuantificó los componentes principales del aceite esencial, a saber, germacrona (25,46 %), xantorizol (17,52 %) y eucaliptol (11,92 %). La actividad larvicida de la nanoemulsión de aceite esencial para *Aedes aegypti* cuantificó una CL50 de 25,94 mg. $L^{-1,}$ siendo definido por el criterio de evaluación como activo en el control y combate de *A. aegypti*. La nanoemulsión esencial de *C. xanthorrhiza* tiene una actividad larvicida eficiente, por lo que se necesitan más estudios sobre su forma de funcionamiento y seguridad para el combate y control de larvas de *Aedes aegypti*.
Palabras clave: *Aedes*; Nanoemulsion; Aceite esencial.

1. Introduction

The *Aedes (*Stegomyia*) aegypti* (Linnaeus, 1762*)* (Diptera:Culicidae*)* is the vector of several diseases, among them yellow fever and dengue, which are a tropical and endemic disease both in South America and in countries with a tropical climate, becoming a worrying problem for health and international interest (Zara et al., 2016; Botas et al., 2017). New forms of control are sought that eliminate the places where the larvae of the *Aedes aegypti* mosquito develop, and that cause less impact to the environment and the population, as well as to prevent the resistance that is created in the vectors, due to to the use of synthetic chemicals such as temephos organophosphate insecticides , natural products being an efficient and ecologically correct alternative as they are of plant origin and biodegradable (Silva, 2006; Ghosh et al., 2013).

In natural products, there are plants that produce as secondary metabolites essential oils, hydrophobic liquids of biological basis, consisting of complex and volatile molecules whose bioactivity depends on the concentration of specific

compounds (Ghosh et al., 2013; Pavoni et al., 2020), which can be extracted from the most diverse parts of a plant, having a wide spectrum of chemicals in various tissues above and below the ground (Miresmailli&Isman, 2014; Sarto&Junior, 2014). In addition to acting in the communication and defense against phytopathogens, they are responsible for the specific flavor and aromas of aromatic species. Thus, essential oils have attracted the interest of the industry and have been considered for the development of plant protection products (Pavela&Benelli, 2016).

However, essential oils, despite having the most diverse biological activities, have limitations of use due to low miscibility and intrinsic water solubility, high volatility and lability, oxidative and hydrolysis facility depending on the structures of their compounds (Pavela&Benelli, 2016; Botas et al., 2017). Limitations that can result in a reduction or even loss of effectiveness of bioactive actions, being a challenge for product development.

The use of nanotechnology has been shown to be a means of overcoming such limitations, which also promote the increase of the biological actions of essential oils and the modulation of the release of active ingredients at the target site, through strategies that involve the creation and use of of different systems on nanometric scales consisting in the development of suitable formulations such as nanoemulsions (Duarte et al., 2015; Faustino et al., 2020; Pavoni et al., 2020), which are isotropic colloidal dispersion systems in which 2 immiscible liquids are dispersed and stabilized by a surfactant, characterized by being thermodynamically stable, and may be one of the means of distribution of bioactive compounds (Shah et al., 2010; Barradas, 2020).

Among the plants that produce essential oils with biological potential and with scarce studies, as for the other parts of its plant, *Curcuma xanthorrhiza* Roxb stands out. Roxburgh called ginger-java, Javanese, or saffron-Javanese (Keiko Izumi et al., 2021). A plant native to Indonesia locally called “Tempulawak”, which is also cultivated in Southeast Asian countries, and belongs to the Zingiberaceae family known also as the ginger family (Rahmat et al., 2021). It is widely used as a raw material for traditional medicine (Justina et al., 2019), also used in cooking as a condiment (Jantan et al., 2012). The rhizome of this plant is an important ingredient for the formulation of jamu, a tonic widely used in Indonesia, and as a stomach drug in Europe (Rohaeti et al., 2014; Cho et al., 2017).

Several authors have reported the effects or pharmacological properties of this plant, such as antioxidant, anti-inflammatory, gastroprotective, neuroprotective and antibacterial (Lukitaningsih, 2020; Kim et al. 2014; Lim et al. 2005; Rahim et al., 2014; Lee et al., 2014). Therefore, we carried out an experimental study to determine the larvicidal activity against Aedes aegypti of the non-emulsion (O/W) of essential oil extracted from the leaves of *Curcuma xanthorrhiza*.

2. Methodology

2.1 Collection of plant material

The samples of *Curcuma xanthorrhiza* leaves were collected in the morning hours in the city of São Luís-MA. After collection, the 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 (EO).

2.2 Essential oil extraction

To extract the essential oil, the hydrodistillation technique was used with a glass Clevenger extractor coupled to a round-bottomed flask packed in an electric blanket. 100 g of ground plant material were used, adding distilled water (1:10). 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 were stored under refrigeration at 4°C. Subsequently, submitted to analysis.

2.3 Gas chromatography coupled to mass spectrometry

The constituents of the essential oil were identified by Gas Chromatography Coupled with 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) (equivalent DB-5MS 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°C (5.0 min.) to 240°C at a rate of 4°C min^{-1} , from 240°C to 300°C (7.5 min) at a rate of 8°C min^{-1}); tT = 60.0 min; Detector: EM; EI (70 eV); Scan mode (0.5 sec $scan^{-1}$); Mass Range: 40-500 daltons (one); Transfer line: 280 ºC.; Filament: off 0.0 to 4.0 min; Linear quadrupole mass spectrometer. To identify the compounds in the sample, the program AMDIS (Automated Mass spectral Deconvolution Mass & Identification System) was used.

2.4 Preparation of nanoemulsions (O/W)

The preparation of nanoemulsions was carried out according to the adapted methodologies described by Lima et al. (2020), Sugumar et al. (2014), Kubitschek et al. (2014) and Rodrigues et al. (2014). The oil-in-water nanoemulsion was formulated with each oil, non-ionic surfactant (Tween 20) and water. The required amounts of each constituent of the oil phase (oil+Tween20) were heated to 65 ± 5°C. The aqueous phase was heated separately to 65 ± 5°C, providing a primary formulation, by the phase inversion method.

To prove stability, the formulated emulsion was subjected to different stress tests (Shafiq et al., 2007). Heating-cooling cycle: it was performed keeping the formulated nanoemulsion at 40 and 4 °C, alternating each temperature for 48 h. The cycle was repeated three times. Freeze-thaw stress: nanoemulsion alternatively at -21 and 25 °C for 48 h at each temperature. The cycle was repeated three times. The formulations that passed the thermodynamic stress tests were taken for larvicidal action studies.

2.5 Larvicidal activity

The eggs were collected in São Luís/MA, through traps called ovitraps. These consist of brown buckets (500 mL), made of polyethylene, with 1 mL of brewer's yeast and 300 mL of running water and two Eucatex straws inserted for mosquito oviposition. The traps were inspected weekly to replace the straws and collect the eggs and sent them to the Laboratory for Research and Application of Essential Oils (LOEPAV/UFMA) of the Technological Pavilion of the Federal University of Maranhão – UFMA.

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

Assays for larvicidal activity were performed according to the adapted methodology proposed by Silva (2006). Initially, a stock solution of 100 mg L^{-1} of each of the essential oils was prepared and diluted in a 2% DMSO solution and nanoemulsions (no dilution). From this solution, five dilutions were prepared at concentrations 1.0-90.0 mg L^{-1}. At each concentration, 10 larvae were added at a rate of 1 mL per larva.

All tests were performed in triplicates and a 2% DMSO solution was used as a negative control, and a 70% v/v ethanol (P.A) solution was used as a positive control. After 24 hours, the live and dead were counted, being considered dead, the larvae that did not react to touch after 24 hours of the beginning of the experiment. To quantify the efficiency of essential oils and nanoemulsions, the Probit statistical test (Finney, 1952) and action classification by Cheng et al. (2003).

3. Results and Discussion

3.1 Chemical constituents

The essential oil extracted from the leaves of *Curcuma xanthorrhiza* presented as major constituents the sesquiterpenes oxygenated germacrone (25.46%) and xanthorrizole (17.52%), followed by the oxygenated monoterpene eucalyptol (11.92%), identified and quantified by GC/MS.

The main components of the essential oil of *C. xanthorrhiza*, cultivated in São Luís-MA, identified in this study are similar to those presented by Sahoo et al. (2022), who when analyzing the essential oil of the leaves of *C. xanthorrhiza* by Gas Chromatography coupled to Mass Spectrometry (GC/MS), cultivated in India, identified a total of 53 constituents, also finding germacrone as the majority in 18.76%, followed by xanthorrizole (12.22%), germacrene B (7 .19%), eucalyptol (6.89%), γ-eudesmol acetate (6.33%), arcurcumene (6.06%), β -himacalene (5.75%) and curzerene (5.24%), and diverge from the presented by Jantan et al. (2012) in which the results showed as main constituents xanthorrizole (31.9%), *β*-curcumene (17.1%), ar- curcumene (13.2%) and camphor (5.4%) for the oil. essential oil obtained from fresh rhizomes of *C. xanthorrhiz* .

In the study proposed by Septama et al. (2022) using the rhizomes of *C. xanthorrhiza* that were collected in Skabumi, West Java in Indonesia the EO showed 42 chemical constituents in the GC/MS analysis, in which a remarkably high amount of α-curcumene and β-curcumene was observed, with percentages of 22.11 and 23.39%, respectively, with curzerene (6.02%), camphor (4.98%) and xanthorrizole (4.65%) also present in considerably high proportions, while Fitria et al. (2019) identified 8 major chemical components in the EO of *C. xanthorrhiza* extracted from rhizomes cultivated in Bog, West Java, Indonesia, namely xanthorrizole (26.77%), α-curmene (15.05%), *β*-curmene (17.04%), camphor (9.06%), epicurzerenone (5.97%), germacrone (5.43%), curzerene (3.90%) and germacrene B (2.08%), thus demonstrating that the EO of this species is composed mostly of terpene compounds.

The differences in relation to the essential oil in this study and in other literatures are expected, as the chemical components contained in the plants can vary significantly, due to different geographic conditions that include soil, climate and seasonal patterns, as well as the conditions of growth and stages of development. maturation to post-harvest processing methods and extraction time (Jantan et al., 2012; Septama et al 2022). And while there have been some variations in the composition and levels of individual constituents of the oils, it is observed that sesquiterpene xanthorrizol is present in this species, both in leaves and rhizomes, suggesting the existence of chemical varieties, but confirming what was reported by Rahmat et al. (2021), that the presence of xanthorrhizol in *C. xanthorrhiza* is species-dependent, thus distinguishing *C. xanthorrhiza* from *C. longa*. This also corroborates the results obtained in this work.

3.2 Larvicidal activity

Table 1 shows the 50% Lethal Concentrations referring to the action of the nanoemulsion against *Aedes aegypti.*

Table 1 - Larvicidal activity of essential oil nanoemulsion against *Aedes aegypti.*

Concentration (mg. L^{-1})	**LC_{50} Mg. L $^{-1}$**	**δ**	**χ^2**	**R^2**
100	25.94 (16.12 - 32.17)	0.222	0.989	0.966
75				
50				
25				
10				

Note: δ-standard desviation; X^2 – chi-square; R^2- correlation coefficient; Source: Authors (2022).

As observed in Table 1, the nanoemulsion of OE from *C. xanthorrhiza* obtained a LC_{50} of 25.94 mg L^{-1} against the *Aedes aegypti* mosquito larvae, demonstrating an activity with high potential, being classified as active because it has a LC_{50} < 50 mg L^{-1} according to the evaluation criteria indicated by Cheng et al. (2003).

The larvicidal action pointed to the analyzed species was also observed by Jantan et al. (2003) who, when verifying the larvicidal activity of the EO of the rhizomes of *Curcuma xanthorrhiza*, using 4th instar *Aedes aegypti* larvae, found a LC_{50} of 74.2 mg L^{-1}, which is a higher value than that obtained in this study. In another investigation carried out by Suilowati et al. (2021), the 70% ethanoic extract of *Curcuma domestica* which is part of the Zingiberaceae family, the same as the raw material under study, had a LC_{50} of 2.084 g L^{-1}.

Ferreira et al. (2022) when analyzing the larvicidal activity of *Coleus aromaticus* Bento against *Aedes aegypti* larvae, obtained a LC_{50} of 3.24 mg L^{-1} for the essential oil and a LC_{50} of 1.83 mg L^{-1}, corroborating that the use of nanoemulsions as a biotechnological product improves the larvicidal action of natural products. Nanoemulsions of other species are reported in the literature, which are also focused on the larvicidal action against *Aedes aegypti*, however they show a lower action potential, for example, the nanoemulsion of *Hyptis suaveolens* of EO with LC_{50} of 202.66 ppm (Silva, 2017) and nanoemulsion of OE from *Ocimum basilicum* Linn had a LC_{50} of 42.15 mg L^{-1} (Santos, 2018).

The results can be justified due to the chemical constituents present in *C. xanthorrhiza* oil, such as eucalyptol and germacrone, which are described in the literature as having good larvicidal activity (Hung et al., 2020; Amado et al., 2020). However, xanthorrizol has not been described in studies associated with larvicidal potential, but covers a wide range of biological potentials such as antibacterial, antiseptic and antioxidant (Salleh et al., 2016). still, the bioactive effects can come from the synergistic interactions of the constituents present in the oil, thus leading to the larvicidal effect, since the terpenic constituents, alcohols and the aldehydes of the EO are mainly responsible for the larvicidal and insecticidal activity of a plant. (Mendes, 2012; Mendes et al., 2017; Dos Santos et al., 2020).

It is important to note that to date, no scientific record on nanoemulsion (O/W) of *C. xanthorrhiza* by the low-energy inversion method is available, however, studies prove the action of nanoemulsions based on essential oils with excellent larvicidal potential (Bricarello et al., 2021; Kavallieratos et al., 2022).

4. Conclusion

Essential oils have shown promise for mosquito control, especially for *Aedes aegypti* species. In this study, the chemical characterization of the essential oil from the leaves of *Curcuma xanthorrhiza* was carried out, which contains the most abundant germacrone (25.46%). An essential oil-laden nanoemulsion was formulated and optimized using a low-energy method, which further simplifies and enhances the processes of amplifying biological effects. The nanoemulsion showed a potent larvicidal effect against *Aedes aegypti* with LC50 25.94 mg. $L^{-1.}$ Thus, this new bioactive nanoemulsion appears as a promising product that can be further investigated in studies regarding its efficacy and safety for combating and controlling *Aedes aegypti* larvae.

Acknowledgments

To the Laboratory for Research and Application of Essential Oils (LOEPAV-UFMA).

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