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Molluscicidal activity against *Biomphalaria glabrata* of the bioactive nanoemulsion of the essential oil of *Thymus vulgaris* Linn (Thyme)

Atividade moluscicida frente *Biomphalaria glabrata* da nanoemulsão bioativa do óleo essencial de *Thymus vulgaris* Linn (Tomilho)

Actividad molusquicida frente a *Biomphalaria glabrata* de la nanoemulsión bioactive del aceite esencial de *Thymus vulgaris* Linn (Tomillo)

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Abstract
This study evaluated the molluscicidal activity against the schistosomiasis-transmitting snail (*Biomphalaria glabrata*) of the nanoemulsion of *Thymus vulgaris* L. essential oil. The plant material was collected in the municipality of São Luís (MA). The essential oil was extracted by hydrodistillation at 100 °C for 3h, with chemical characterization performed by Gas Chromatography coupled to Mass Spectrometry (GC-MS). The nanoemulsions were prepared using the phase inversion method. For molluscicidal activity, the methodology recommended by the Who was performed, being the LC_{50} of the essential oil nanoemulsion for their action against the snail obtained by the Probit method. The major constituents found in *Thymus vulgaris* L. essential oil were carvacrol (27.35%) and thymol (24.12%). The molluscicidal activity of the essential oil nanoemulsion presented an LC_{50} of 15.04 mg L^{-1}. The results indicate that the essential oil nanoemulsion evaluated is composed of substances that provide and encourage its application, due to its high potential for molluscicidal activity against *Biomphalaria glabrata*.
Keywords: Molluscicidal; Nanoemulsion; Essential oil; Schistosomiasis.

Resumo
Este estudo avaliou a atividade moluscicida frente ao caramujo transmissor da esquistossomose (*Biomphalaria glabrata*) da nanoemulsão do óleo essencial de *Thymus vulgaris* L. O material vegetal foi coletado no munícipio de São Luís (MA). O óleo essencial foi extraído por hidrodestilação a 100 °C por 3h, com caracterização química realizada através de Cromatografia Gasosa acoplada a Espectrometria de Massas (CG-EM). As nanoemulsões foram preparadas através do método de inversão de fases. Para atividade moluscicida executou-se a metodologia preconizada pela OMS, sendo a CL_{50} da nanoemulsão do óleo essencial para ação dos mesmos frente ao caramujo obtido pelo método de Probit. Os constituintes majoritários encontrados no óleo essencial de *Thymus vulgaris* L. foram o carvacrol (27,35%) e o timol (24,12%). A atividade moluscicida da nanoemulsão do óleo essencial apresentou a CL_{50} de 15,04 mg L^{-1}. Os resultados indicam que a nanoemulsão do óleo essencial avaliado é composta por substâncias que propiciam e incentivam sua aplicação, devido ao seu alto potencial para atividade moluscicida frente *Biomphalaria glabrata*.
Palavras-chave: Moluscicida; Nanoemulsão; Óleo essencial; Esquistossomose.

Resumen
El estudio evaluó la actividad molusquicida contra el caracol transmisor de la esquistosomiasis (*Biomphalaria glabrata*) de la nanoemulsión de aceite esencial de *Thymus vulgaris* L. El material vegetal fue recolectado en el municipio de São Luís (MA). El aceite esencial se extrajo por hidrodestilación a 100 °C por 3h, con caracterización química realizada por Cromatografía de Gases acoplada a Espectrometría de Masas (GC-MS). Las nanoemulsiones se prepararon mediante el método de inversión de fase. Para la actividad molusquicida se realizó la metodología recomendada por la OMS, siendo la CL_{50} de la nanoemulsión de aceites esenciales para su acción contra el caracol obtenida por el método Probit. Los principales componentes encontrados en el aceite esencial de *Thymus vulgaris* L. fueron carvacrol (27,35%) y timol (24,12%). La actividad molusquicida de la nanoemulsión de aceite esencial presentó una CL_{50} de 15,04 mg L^{-1}. Los resultados indican que la nanoemulsión de aceite esencial evaluada está compuesta por sustancias que brindan y favorecen su aplicación, debido a su alto potencial de actividad molusquicida frente a *Biomphalaria glabrata*.
Palabras clave: Molusquicida; Nanoemulsión; Aceite esencial; Esquistosomiasis.

1. Introduction

Schistosomiasis is a disease characteristic of the tropical region. In Brazil, through work and research, the pathology is direct to regions where there is the presence of the transmitting mollusks. Generally, the people who are most at risk of contracting the pathology are those who reside on river or lake slopes, in which they are not usually well assisted by the public authorities in terms of health treatments (Silva et al., 2014).

Schistosomiasis, caused by the trematode *Schistosoma mansoni* is one of the relevant endemic diseases not only in Brazil, but in several tropical countries. The reproductive life cycle is characterized by the presence of an intermediate host (the snail) and the definitive host (man), in Brazil it is classified as snails of the genus *Biomphalaria,* referring to the snail *B. glabrata* as the main vector of the Americas from the South and Central (Carvalho et al., 2008).

Recently, for synthetic molluscicidal activities in order to inhibit the action by the snail, niclosamide is used as a control tool in relation to the snail (Cantanhede et al., 2010). Despite offering action to combat snails at low concentrations, equivalent to 1 mg L^{-1}, however, they cause a series of implications for the environment and resistance of *B. glabrata* molluscs. However, as much as they show positive results, it is necessary to search for biodegradable molluscicide, that is, having plants as a source, becoming the best alternative for the environment and less toxicity (Colley et al., 2014).

In this context, the use of herbal medicines has intensified in recent years, considering that the use of natural products has significantly mitigated the environmental impacts caused by the use of synthetic products. In addition, there is a high diversity of species that can be applied, and their rapid degradation and especially at the most affable cost (Coêlho et al., 2013). The development of nanostructured bioproducts such as nanoemulsions made from essential oils stand as alternatives to overcome barriers and limitations that oils present (Araujo et al., 2021; McClements et al., 2021; Garcia et al., 2022).

The positive expectations directed at solutions based on biological activities of plant metabolism products, based on secondary metabolites, are justified by their intense chemical complexity. These factors can be proven in some studies focusing on essential oils, such as: antioxidant activity (Costa et al., 2021), larvicidal (Santos et al., 2020), antibacterial (Furlani et al., 2021), anti- inflammatory (Melo et al., 2021), molluscicidal (Salama et al., 2012), attesting to its diversified potential.

In view of the above, there is the species of Thymus vulgaris L., known as "garden thyme", which belongs to the *Lamiaceae* family, used since ancient times in folk and culinary medicine, as well as in industry, with its commercial use being explored. increasingly, although studies involving specific parts of this plant are still scarce, as the aerial parts the literature describes about the good potential of biological action presented by the essential oil of T. vulgaris (Patil et al., 2021; Galovičová et al., 2021).

Thus, this study aimed to evaluate the molluscicidal activity against *Biomphalaria glabrata* (schistosomiasis-transmitting snail) of the bioactive nanoemulsion of *Thymus vulgaris* L. essential oil.

2. Methodology

2.1 Collection of plant material

Thymus vulgaris leaf samples were collected in the morning hours in 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

For the extraction of 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 μ L 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. Formulations that passed thermodynamic stress tests were taken to further studies.

2.5 Molluscicidal activity

For the evaluation of molluscicidal activity, the technique recommended by the World Health Organization (1983) was applied, in which two tests are performed. In the first, called pilot test, a solution of the nanoemulsion under study was prepared in a volume of 500 mL at a concentration of 100 mg L^{-1}, where 10 adult snails *Biomphalaria glabrata*, negative for *Schistosoma mansoni*, were inserted, obtaining in the final a ratio of 50 mL/snail and fed with hydroponic lettuce. They were exposed in 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.

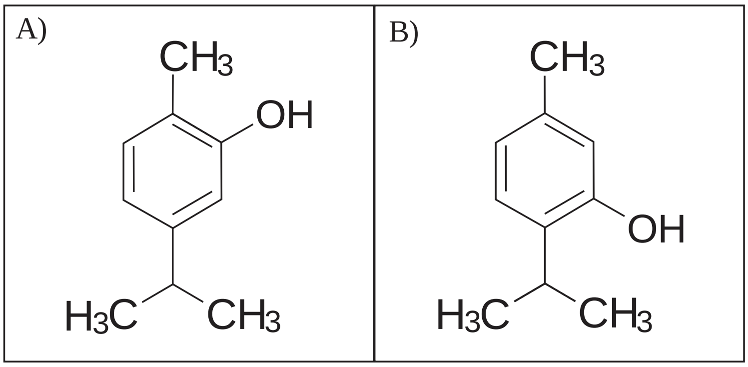
In the second test, the Lethal Concentration ($LC_{50)}$ was evaluated, where essential oil/nanoemulsion solutions were prepared in a volume of 500 mL at concentrations of 100, 80, 60, 40, 20, 10 and 5 mg L^{-1}, using the same pilot test methodology. Positive, negative and blank controls were also performed. Mortality rates were obtained as the mean number of dead individuals as a function of the logarithm of the dose tested. The statistical analysis of the data for the LC_{50} was performed according to the Probit method (Finney, 1952).

3. Results and Discussion

3.1 Chemical constituents

According to the GC/MS, it is possible to highlight carvacrol (27.35%) as the major compound, followed by thymol (24.12%).

Figure 1 - Constituent structures A) carvacrol and B) thymol.



Source: Authors.

Similar results were observed in relation to the study by Rocha et al. (2013), in which were analyzed by Gas Chromatography coupled to Mass Spectrometry (GC/MS), thymol (27.65%) and carvacrol (10.09%) respectively, being these classified as oxygenated monoterpenes, emphasizing that these secondary metabolites under different environmental conditions may change in relation to their quantity. The study by Borges et al. (2012), also contributes to the present work, in which the following chemical constituents identified through analysis (GC/MS) stand out, with borneol (66.66%), higher than the thymol content (13.41%) and linalool (3.24%). In view of the aforementioned study, it is confirmed that the monoterpene thymol is one of the major metabolites.

In view of the study proposed by Nunes et al. (2021), the EO *Thymus vulgaris* presented 23 substances that were evaluated by analysis (GC/MS), being carvacrol (26.14%), p-cymene (23.75%) and thymol (21.78%). Other authors also classify these chemical constituents thymol (53.5%), p-cymene (20.8%) and carvacrol (8.9%) as major secondary metabolites (Porto et al., 2021). It is noteworthy that the variations in terms of results obtained by the mentioned studies are due to the fact that the isomers thymol and carvacrol are biosynthesized based on γ - terpinene and ρ-cymene, which contribute to the identification of these compounds (Nunes et al., 2021). In addition, abiotic and biotic factors can significantly modify the biosynthetic composition in plant species, contributing to a greater diversity of secondary metabolites, as a result of exposure to adverse effects and stress (Khalil et al., 2018).

3.2 Characterization of nanoemulsions

The formulated nanoemulsion was characterized as an oil-in-water nanoemulsion and evaluated as a stable formulation. Thus, the average droplet size of this formulation was monitored.

According to the observed result of the nanoemulsion of *Thymus vulgaris* on the first day of formulation manipulation there was a droplet size of 155.80 nm ± 0.25 nm and low polydispersity (0.254 ± 0.002). Thus, observing the nanoemulsion after 30 days, the formulation had a small droplet size (160.11 ± 0.38) and low polydispersity (0.144 ± 0.001), not showing phase separation or any other type of unstable behavior. According to the average droplet size smaller than 200 nm after 1 and 30 days of handling the formulation was considered a nanoemulsion (Bruxel et al., 2012).

3.3 Molluscicide front Biomphalaria glabrata

Table 1 presents the results regarding the molluscicidal activity of the nanoemulsion of *Thymus vulgaris* essential oil against *Biomphalaria glabrata*.

Table 1 - *Biomphalaria glabrata* mortality for nanoemulsion action by the Probit method.

Log C	LC_{50} mg L^{-1}	LC_{90} mg L^{-1}	χ^2	σ	R^2
1.00 **1.30** **1.48** **1.70** **1.78** **1.85** **1.95** **2.00** **2.08**	**15.04** (9.12 - 17.66)	**57.36** (50.14 - 75.25)	0.9999	0.475	0.988

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

In front of Table 1, it is stated that the EO of *Thymus vulgaris* presented a Lethal Concentration 50% (LC_{50}) following the value of 15.04 mg L^{-1} and LC $_{90}$ of 57.36 mg L^{-1}. According to the results obtained, it can be attested that the values are within the standards determined by the Who (1983), defined by the agency, that the most diverse plant species have active molluscicidal effectiveness in concentrations of up to 100 mg L^{-1}).

The present work attested to positive results compared to those of Ribeiro et al. (2016), who verified the molluscicidal activity of EO of *Hyptis dilatata* Benth, which is part of the *Lamiaceae* family, that is, the same as *T. vulgaris* against the snail *Biomphalaria glabrata* and verifying the following quantitative values: LC_{50} and LC_{90} of 112.46 µg mL^{-1} and 182.33 µg mL^{-1} respectively, proving that the results obtained are in accordance with the standards determined by the Who (1983).

It is stated that, for a substance to present molluscicidal activity, it must meet the conditions of eliminating the snail at all stages belonging to its life cycle in areas characterized as its natural habitat, be biodegradable for the benefit of the environment, have applications to lower concentrations, show speciation in terms of lethality to the snail *B. glabrata* and especially not to pose risks to humans (Who, 1983).

It is noteworthy that all the criteria mentioned by (Who, 1983) were respected, proving that the EO of the *T. vulgaris* species is an excellent alternative to replace conventional synthetic products available in the fight against *B. glabrata*.

The molluscicidal activity is linked to secondary metabolites present in the most varied species of plants, among them flavonoids, tannins, saponins, among others. According to the analysis (GC/MS) carried out in the present study, it is speculated that the greatest contribution of thymol and carvacrol occurred, however it is prudent to say that all metabolites present in the EO of the *T. vulgaris* plant contribute to the synergistic effect, to confirm it would be necessary more specific tests in relation to its identified major metabolites (Cantanhede, 2010).

4. Conclusion

Thymus vulgaris L. essential oil proved to be a potential product for application in molluscicidal activities against *Biomphalaria glabrata*, thus characterizing as an alternative for the inhibition of the schistosomiasis vector, proving a low toxicity to humans and for its simplicity in terms of extraction.

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References

Araujo, T. D. S., da Costa, J. M. A. R., Ribeiro, F. D. O. S., de Jesus Oliveira, A. C., do Nascimento Dias, J., de Araujo, A. R., ... & de Souza, B. W. S. (2021). Nanoemulsion of cashew gum and clove essential oil (Ocimum gratissimum Linn) potentiating antioxidant and antimicrobial activity. *International Journal of Biological Macromolecules*, *193*, 100-108.

Borges, A. M., Pereira, J., Cardoso, M. G., Alves, J. A., & Lucena, E. M. P. (2012). Determinação de óleos essenciais de alfavaca (Ocimum gratissimum L.), orégano (Origanum vulgare L.) e tomilho (Thymus vulgaris L.). *Revista Brasileira de Plantas Medicinais*, *14*, 656-665.

Bruxel, F., Laux, M., Wild, L. B., Fraga, M., Koester, L. S., & Teixeira, H. F. (2012). Nanoemulsões como sistemas de liberação parenteral de fármacos. *Química Nova*, *35*, 1827-1840.

Cantanhede, S. P. D., Marques, A. D. M., Silva-Souza, N., & Valverde, A. L. (2010). Atividade moluscicida de plantas: uma alternativa profilática. *Revista Brasileira de Farmacognosia*, *20*, 282-288.

Coêlho, M. D. G., da Silva, V. A. R., Pereira, J. R., Akisue, G., da Silva Coêlho, F. A., & Furtado, F. N. (2013). Avaliação "in vitro" do potencial acaricida do óleo essencial de Tagetes minuta frente a Riphicephalus (Boophilus) microplus (Canestrini, 1887). *Revista Biociências*, *19*(1).

Colley, D. G., Bustinduy, A. L., Secor, W. E., & King, C. H. (2014). Human schistosomiasis. *The Lancet*, *383*(9936), 2253-2264.

Costa, K. P., Fonseca, E. S., Andrade, R. E. S., Ferreira, G. S., Rodrigues, L. I. T., da Fonseca, F. S. A., & Martins, E. R. (2021). Atividade antioxidante dos extratos etanólicos e dos óleos essenciais de Xylopia aromática e Piper nigrum. *Brazilian Journal of Development*, *7*(3), 27904-27912.

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

Furlani, R., Sousa, M., Rocha, G. N. D. S. A., Vilar, F. C. R., Ramalho, R. C., & Peixoto, R. D. M. (2021). Atividade antibacteriana de óleos essenciais frente aos patógenos de importância na mastite caprina e ovina. *Revista Caatinga*, *34*(3), 702-708.

Galovičová, L., Borotová, P., Valková, V., Vukovic, N. L., Vukic, M., Štefániková, J., ... & Kačániová, M. (2021). Thymus vulgaris essential oil and its biological activity. *Plants*, *10*(9), 1959.

Garcia, C. R., Malik, M. H., Biswas, S., Tam, V. H., Rumbaugh, K. P., Li, W., & Liu, X. (2022). Nanoemulsion delivery systems for enhanced efficacy of antimicrobials and essential oils. *Biomaterials Science*, *10*(3), 633-653.

Khalil, N., Fekry, M., Bishr, M., El-Zalabani, S., & Salama, O. (2018). Foliar spraying of salicylic acid induced accumulation of phenolics, increased radical scavenging activity and modified the composition of the essential oil of water stressed Thymus vulgaris L. *Plant Physiology and Biochemistry*, *123*, 65-74.

Kubitschek-KM, A. R. J., & Zero, J. M. (2014). Development of jojoba oil (Simmondsia chinensis (Link) CK Schneid.) based nanoemulsions. *Lat. Am. J. Pharm*, *33*(3), 459-63.

Lima, T. C. P., de Almeida, A. F., de Oliveira, E. C. P., Carrera Silva Júnior, J. O., Ribeiro Costa, R. M., Pena Matos, A., & Fonseca Gomes, M. R. (2020). Desenvolvimento de nanogel de copaifera reticulata sobre a lesão muscular em ratos usando fonoforese. *Saúde e Pesquisa*, *13*(1).

McClements, D. J., Das, A. K., Dhar, P., Nanda, P. K., & Chatterjee, N. (2021). Nanoemulsion-based technologies for delivering natural plant-based antimicrobials in foods. *Frontiers in Sustainable Food Systems*, *5*, 643208.

Melo, A. F. M., de Sousa, L. D. F. L., do Nascimento Júnior, W., do Nascimento, W. L., da Costa Tenório, R., da Silva Rodrigues, R. R., ... & Santana, L. S. O. S. (2021). Alecrim (rosmarinus officinalis l.) Atividade anti-inflamatória: uma revisão de literatura. *Revista de Casos e Consultoria*, *12*(1), e24346-e24346.

Nunes, C. R., Valente, P. M., da Silva, F. D., & Valente, V. M. M. (2021). Composição química e atividade antifúngica do óleo essencial de Thymus Vulgaris sobre Aspergillus Niger, Penicillium Expansum, Sclerotinia Sclerotiorum E Sclerotium Rolfsii. *Brazilian Journal of Development*, *7*(2), 14250-14260.

Patil, S. M., Ramu, R., Shirahatti, P. S., Shivamallu, C., & Amachawadi, R. G. (2021). A systematic review on ethnopharmacology, phytochemistry and pharmacological aspects of Thymus vulgaris Linn. *Heliyon*, *7*(5), e07054.

Porto, M., Valadares, Y., Silva, C., Santos, R., & Gualberto, S. (2021). Avaliação do potencial antioxidante do óleo essencial de Thymus vulgaris L. (Lamiaceae). *Enciclopédia Biosfera*, *18*(38).

Ribeiro, S. M., Bonilla, O. H., & Lucena, E. M. P. (2018). Influência da sazonalidade e do ciclo circadiano no rendimento e composição química dos óleos essenciais de Croton spp. da Caatinga. *Iheringia, Série Botânica.*, *73*(1), 31-38.

Rocha, B. C. A. D. (2013). *Extração e caracterização do óleo essencial de tomilho (Thymus vulgaris)* (Tese de mestrado, Universidade Federal Rural do Rio de Janeiro).

Rodrigues, E. D. C., Ferreira, A. M., Vilhena, J. C., Almeida, F. B., Cruz, R. A., Florentino, A. C., ... & Fernandes, C. P. (2014). Development of a larvicidal nanoemulsion with Copaiba (Copaifera duckei) oleoresin. *Revista Brasileira de Farmacognosia*, *24*, 699-705.

Salama, M. M., Taher, E. E., & El-Bahy, M. M. (2012). Atividades moluscicida e mosquitocida de óleos essenciais de Thymus capitatus Hoff. et Link. e de Marrubium vulgare L. *Revista do Instituto de Medicina Tropical de São Paulo*, *54*(5), 281-286.

Santos Carvalho, O., Coelho, P. M. Z., & Lenzi, H. L. (Eds.). (2008). *Schistosoma mansoni & Esquistossomose: uma visão multidisciplinar*. SciELO-Editora FIOCRUZ.

Santos, A. B. D. S., Everton, G. O., Júnior, R. G. D. O. C., Rosa, P. V. S., Pereira, A. P. M., dos Santos Souza, L., ... & Mouchrek Filho, V. E. (2020). Óleos essenciais de Cinnamomum zeylanicum Blume e Plectranthus amboinicus (lour.) Spreng como agentes larvicidas frente as larvas do Aedes aegypti. *Brazilian Journal of Development*, *6*(4), 22355-22369.

Shafiq, S., Shakeel, F., Talegaonkar, S., Ahmad, F. J., Khar, R. K., & Ali, M. (2007). Development and bioavailability assessment of ramipril nanoemulsion formulation. *European journal of pharmaceutics and biopharmaceutics*, *66*(2), 227-243.

Silva, A. G. D. S., França Junior, D. B., Santos, H. F. D. A. C., Moura, H. L. S., Garcia, P. T., Lima, C. S. D. C., ... & Moreira, J. C. R. (2014). Doenças transmissíveis: doenças negligenciadas associadas à pobreza e a vigilância em saúde.

Sugumar, S., Clarke, S. K., Nirmala, M. J., Tyagi, B. K., Mukherjee, A., & Chandrasekaran, N. (2014). Nanoemulsion of eucalyptus oil and its larvicidal activity against Culex quinquefasciatus. *Bulletin of entomological research*, *104*(3), 393-402.