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MOLLUSCICIDAL ACTIVITY OF CRUDE EXTRACTS OF THE SPECIES *MORINGA OLEIFERA* LAM. ON *BIOMPHALARIA GLABRATA* (SAY, 1818)

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Abstract: Schistosomiasis is an infectious-parasitic disease caused by trematode worms of the species *Schistosoma mansoni*. One of the ways to interrupt the cycle of this disease is through the control or elimination of intermediate hosts. Chemical molluscicide is one of the approaches used in preventing this disease. The WHO recommends the use of the synthetic molluscicide Niclosamide, however, low selectivity for *B. glabrata*, toxicity to non-target organisms and the environment. Due to the aforementioned scenario, the search for products of natural origin that are efficient as molluscicidal substances began. *Moringa oleifera* Lam. belongs to the Moringaceae family, has some pharmacological properties, such as hepatoprotective, antidiabetic activity, carcinogenic, antioxidant and antimicrobial activity. The objective of this work was to evaluate the molluscicidal activity of crude extracts in hexane, dichloromethane, ethyl acetate, chloroform, acetone, ethanol, methanol and butanol against the species: *Biomphalaria glabrata*. The leaves of the *Moringa oleifera* species were extracted by exhaustion in a soxhlet extractor with organic solvents, hexane, dichloromethane, chloroform, ethyl acetate, acetone, ethanol, methanol and butanol to obtain crude extracts. The experiments were carried out in 24-well plates, using the methodology of SANTOS et al. (2017). It was possible to observe that the crude extracts of the leaves of the species *Moringa oleifera* showed molluscicidal activity on the species *Biomphalaria glabrata*. Among the aforementioned extracts, the crude extract in dichloromethane and the crude extract in hexane showed better activity on the intermediate host. The crude extract in dichloromethane showed a lethality of 100% at a concentration of 100 mg/L ($p = 0.0372$) within 48 h and the crude extract in hexane showed a mortality of 100% at a concentration of 100 mg/L ($p = 0, 0418$) in 48 h. Despite the concentration not being in accordance with that recommended by the World Health Organization (WHO), these extracts present a possibility as an alternative in population control in the vector of schistosomiasis disease.

Key words: Natural products, *Moringa oleifera*, schistosomiasis, biological activity, *Biomphalaria glabrata*.

INTRODUCTION

Schistosomiasis is an infectious-parasitic disease caused by trematode worms of the species *Schistosoma mansoni* (GOMES *et al*., 2021; SALEH *et al*., 2022, SOUZA *et al*., 2022). This disease is directly related to a major public health problem, affecting less economically favored places, without access to adequate basic sanitation (BARRETO and LOBO, 2021).

This neglected disease is typical in regions such as Brazil, North and South America, Africa and Asia regions. Data from the World Health Organization (WHO) estimate that 258 million people in 78 countries around the world are infected by the disease, 52 countries with moderate and high transmission. Approximately 700 million people live in endemic areas (WHO, 2022).

This parasitic disease is popularly known as "water belly" and "snail disease" (SOUZA *et al*., 2021). The cycle of this disease is directly related to the intermediate host, being molluscs, and the definitive host, being man, for transmission to occur. In Brazil, the species *Biomphalaria glabrata* is the most important intermediate host, due to its wide geographic distribution and high levels of infection (RIBEIRO *et al*., 2021).

One of the ways to interrupt the cycle of this disease is through the control or elimination of intermediate hosts. Chemical molluscicide is one of the approaches used to prevent this parasitic disease (IBRAHIM et al., 2021). The

WHO recommends the use of the synthetic molluscicide Niclosamide, commercially known as Bayluscide®. However, this product has a high operating cost, low selectivity for the *B. glabrata* species, toxicity to non-target organisms and the environment (MTEMELI *et al*.; 2021; MINISTÉRIO DA SAÚDE, 2008).

Due to the aforementioned scenario, the search for products of natural origin, derived from plants, that are efficient as molluscicidal substances began. This alternative product must be more selective for the target species, non-toxic to non-target animals and the environment (MINISTÉRIO DA SAÚDE, 2008).

Moringa oleifera Lam. It belongs to the Moringaceae family, a fast-growing and drought-resistant tree. This species is popular as it adapts to different environments. It is popularly known as moringa, horseradish and miracle tree (ANZANO *et al*., 2021).

According to the literature, this species has some pharmacological properties, such as hepatoprotective, antidiabetic activity, carcinogenic, antioxidant and antimicrobial activity. In addition, it is also used in the treatment of people with malnutrition and aid in breastfeeding, in the improvement of breast milk production (ANZANO *et al*., 2021; SHARMA *et al*., 2022).

According to Padayachee and Baijnath (2020), this species already has its secondary metabolites described. In the different parts of the plant, it is possible to find alkaloids, flavonoids, carotenoids, anthraquinones, tannins, anthocyanins and proanthocyanidins. Possibly, these described secondary metabolites contribute to the pharmacological properties.

Thus, the present study aimed to evaluate the molluscicidal activity of crude extracts in hexane, dichloromethane, ethyl acetate, chloroform, acetone, ethanol, methanol and butanol against the species *Biomphalaria glabrata*.

MATERIAL AND METHOD

PLANT USED

Moringa oleifera Lam. was collected in July 2010, at the Oswaldo Cruz Foundation Campus - Fiocruz (22º52'33"S 43º14'46"W), RJ, Brazil. Identification and classification were duly carried out in Jardim Botânico in Rio de Janeiro, RJ, by Dr. Ray Harley of the Royal Botanical Gardens at Kew. The identified species is deposited in the herbarium of the Botanical Garden of Rio de Janeiro, under the number RB498458.

PREPARATION OF CRUDE EXTRACTS

The crude extracts were prepared at the Laboratory for the Evaluation and Promotion of Environmental Health at Instituto Oswaldo Cruz – Fiocruz.

The leaves of the *Moringa oleifera* species were dried in a forced ventilation oven and ground in a blender (15 g of powder) were extracted by exhaustion in a soxhlet extractor with organic solvents, hexane, dichloromethane, chloroform, ethyl acetate, acetone, ethanol, methanol and butanol to obtain the crude extracts.

The crude extracts were concentrated in a rotary evaporator and transferred to previously tared and identified flasks. These flasks were placed for evaporation in a chemical exhaust hood and weighed daily until constant weight was obtained.

CREATION AND MAINTENANCE OF MOLLUSCS

The molluscs of the species *Biomphalaria glabrata*, collected in Sumidouro, Rio de Janeiro, RJ, Brazil, (S 22º 02' 59" and W 42º 40' 29"), used in the experiments were bred, kept in breeding tanks with a capacity of 200 L

with dechlorinated water and fed three times a week with fresh lettuce leaves at the Lauro Travassos Pavilion on the Oswaldo Cruz Foundation Campus, at coordinates S 22º 52' 33" and W 43º 14' 46". In order to obtain and maintain quantities of molluscs that meet the demands of the laboratory, Styrofoam plates, with approximate sizes of 10 cm X 10 cm, are added to obtain embryos.

MOLLUSCIDIAL ACTIVITY TEST

The experiments were carried out in 24-well plates, using the methodology of SANTOS et al. (2017), where adult individuals with a shell diameter of 10-12 mm of the species *B. glabrata*, not infected by *S. mansoni*, were allocated individually in a 24-well plate. The snails were exposed to negative controls (distilled water and 1% DMSO), positive control (Niclosamide® 2 mg/L) and different concentrations of extracts for a final volume of 2 mL (2000 µL) per well. The molluscs were exposed, for 24 h and 48 h, to the concentrations of the different extracts. 21 animals were used for each experiment, in which all tests were performed in triplicate, and at least on 3 different days. Mortality was observed in the period of 24 h and 48 h. Lethality can be identified through the appearance of the shells, lack of mobility, odors, soft tissue retraction and hemolymph release (Mc Cullough et al., 1980).

STATISTICAL ANALYSIS

Statistical analysis of the results was performed by arithmetic mean ± standard deviation. The mean of the groups was analyzed through linear regression using the Graph Pad Prism 5 program. The difference was considered significant when $p < 0.05$.

RESULTS

The yields of the crude extracts obtained were: crude extract in hexane = 11.324%, crude extract in dichloromethane = 9.293%, crude extract in ethyl acetate = 32.695%, crude extract in chloroform = 9.577%, crude extract in acetone = 15.997%, crude extract in ethanol = 28.223%, crude extract in methanol = 32.642% and crude extract in butanol = 98.121% (Table I).

Crude extract	Production (%)
Crude extract in hexane	11.324
Crude extract in dichloromethane	9.293
Crude extract in ethyl acetate	32.695
Crude extract in chloroform	9.577
Crude extract in acetone	15.997
Crude extract in ethanol	28.223
Crude extract in metanol	32.642
Crude extract in butanol	98.121

Table I: Yield of crude extracts from leaves of the species *Moringa oleifera* Lam.

In the crude extract in hexane, 100% mortality was observed at a concentration of 100 mg/L in 48 h. At a concentration of 150 mg/L, it presented a lethality of 55. 67% and 100%, in 24 h and 48 h, respectively. Linear regression analysis was performed and presented p value = 0.0418 (Figure 1).

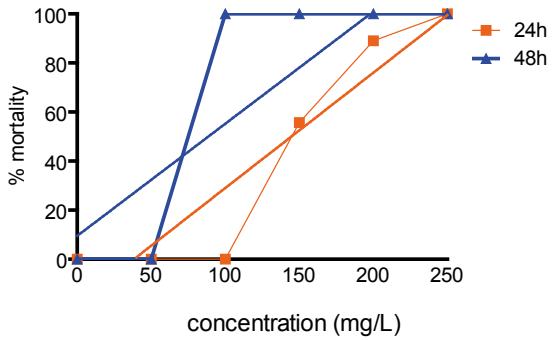

Figure 1: Toxicity of the hexane extract ofMoringaoleifera leaves onBiomphalaria glabrata. n=3

The crude extract in dichloromethane showed a lethality of 11% at a concentration of 50 mg/L in 24 h. At a concentration of 150 mg/L, it was possible to observe a mortality rate of 100% within 24 h. Linear regression analysis was performed and presented p value = 0.0372 (Figure 2).

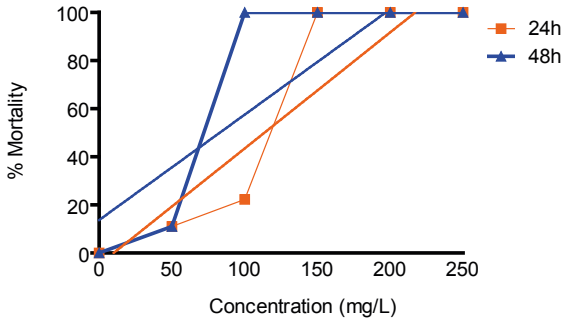

Figure 2: Toxicity of dichloromethane extract fromMoringaoleifera leaves onBiomphalaria glabrata. n=3

The crude extract in ethyl acetate showed a mortality rate of 33.33% at a concentration of 100 mg/L in 48 h. At a concentration of 200 mg/L, it was possible to observe 100% mortality within 24 h. Linear regression analysis was performed and presented p value = 0.0385 (Figure 3).

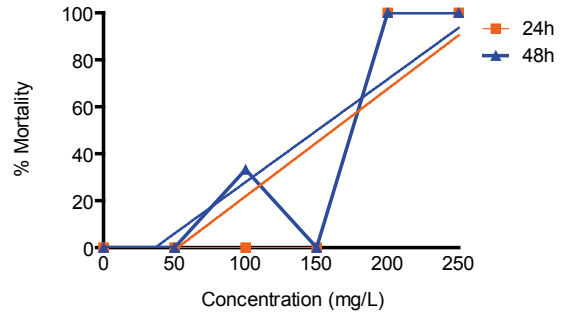

Figure 3: Toxicity of ethyl acetate extract fromMoringaoleifera leaves onBiomphalaria glabrata. n=3

The crude extract in chloroform presented at a concentration of 100 mg/L, lethality of 11% in 24 h and 22.33% in 48 h. From the concentration of 100 mg/L, it was possible to observe 100% lethality in the period of 24 h. Linear regression analysis was performed and presented p value = 0.0109 (Figure 4).

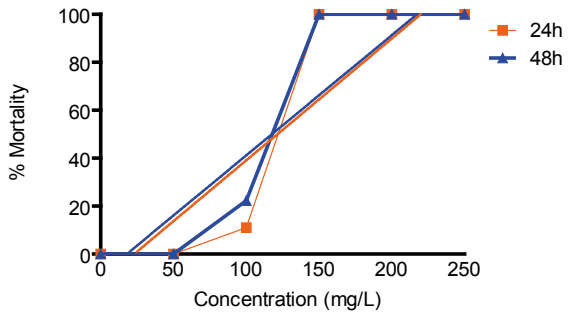

Figure 4: Toxicity of chloroform extract ofMoringaoleiferaleaves onBiomphalaria glabrata. n=3

In the crude extract in acetone, it was possible to observe that the concentration of 100 mg/L presented 22.33% of mortality in 48 h. At a concentration of 150 mg/L, lethality was 89% in 48 h. At a concentration of 200 mg/L, 100% mortality was observed within 24 h. Linear regression analysis was performed and presented p value = 0.0061 (Figure 5).

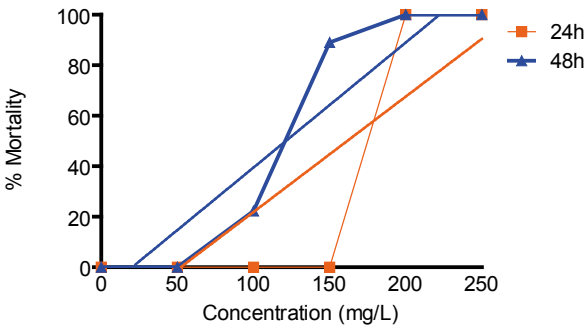

Figure 5: Toxicity of acetone extract of Moringaovalifolia leaves on Biomphalaria glabrata. n=3

In the crude extract in ethanol, mortality started at a concentration of 150 mg/L. It was observed that in this concentration the lethality was 66.67% and 100%, in 24 h and 48 h, respectively. Linear regression analysis was

performed and presented p value = 0.0213 (Figure 6).

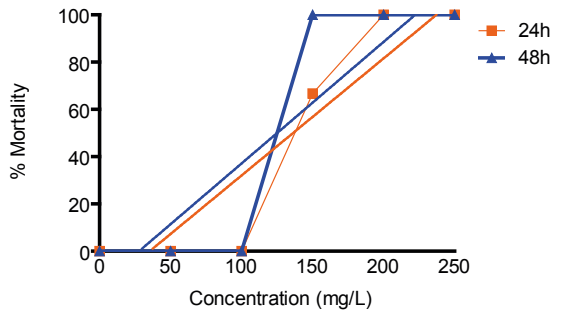

Figure 6: Toxicity of ethanol extract fromMoringa oleiferaleaves onBiomphalaria glabrata. n = 3

In the crude extract in methanol, lethality started at a concentration of 100 mg/L. At a concentration of 150 mg/L, a mortality rate of 44.44% and 100% was observed in 24 h and 48 h, respectively. Linear regression analysis was performed and presented p value = 0.0151 (Figure 7).

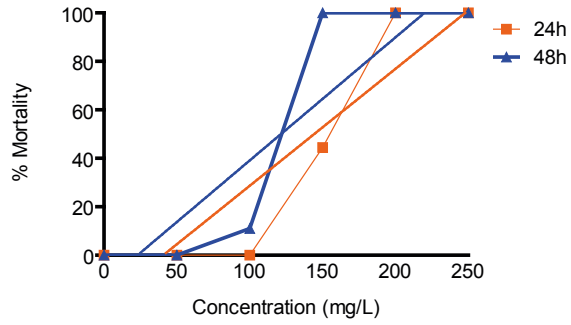

Figure 7: Toxicity of methanol extract ofMoringa oleiferaleaves onBiomphalaria glabrata. n=3

In the crude extract in butanol, at a concentration of 100 mg/L, mortality of 44.33% was observed in 48 h. At a concentration of 150 mg/L, lethality was 55.67% and 100%, in 24 h and 48 h, respectively. Linear regression analysis was performed and presented p value = 0.0081 (Figure 8).

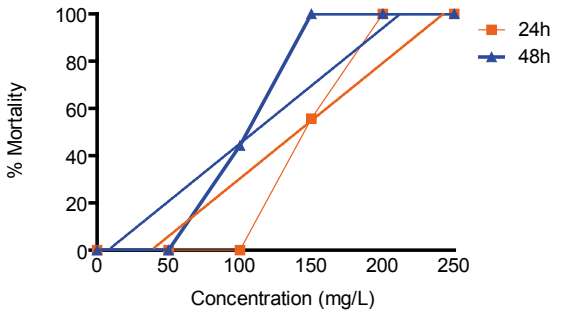

Figure 8: Toxicity of butanol extract fromMoringaoleiferaleaves onBiomphalaria glabrata. n = 3

DISCUSSION

Medicinal plants have been used for many years, through traditional knowledge, becoming common for the treatment and symptoms of some diseases. They are also often used as an alternative treatment for people in areas with less financial resources. There are several studies that prove different pharmacological properties of different plants (MELO *et al.*, 2017).

Plants have a diversity of metabolites that allow them different pharmacological properties and these properties can have different therapeutic effects. In this research we will use the species *Moringa oleifera* Lam. This species is widely used due to its different properties, such as: antidiabetic, anti-inflammatory, antioxidant and anticancer (MA *et al.*; 2018; MELO *et al.*, 2017; MILLA *et al.*, 2021).

Moringa oleifera has been used in India as a medicinal plant since the 18th century BC Traditional healers use all parts of the plant, flowers, leaves, stem, seeds for the treatment of different diseases such as: heart problems, anemia, asthma and bronchitis (MA *et al.*; 2018; MILLA *et al.*, 2021). Our group used the leaves of this species to evaluate the molluscicidal activity of schistosomiasis intermediate host molluscs.

Silva et al. (2013) used M. oleifera seeds

to evaluate the molluscicidal activity of the species *Biomphalaria glabrata, Physa marmorata and Melanoides tuberculatus.* Rocha-Filho et al. (2015) used the extract of the flower of the species M. oleifera to evaluate the bioactivity of molluscs of the species *B. glabrata*, cercarias of *Schistosoma mansoni* and crustaceans of the species *Artemia salina.* Ibrahim and Abdalla (2017) used *M. oleifera* seeds to analyze the molluscicidal activity of the species *Biomphalaria alexandrina*. Nnamdi and collaborators also used the seeds of the species *M. oleifera* to evaluate molluscs of the species *Bulinus*. It is possible to observe the versatility of this plant species to evaluate different biological activities.

David et al. (2016) evaluated the mortality of embryos of the species *Danio rerio* (Zebrafish) that were exposed to the extract of the leaves of the species *M. oleifera*. According to the results obtained, it was possible to observe that in the concentration of 300 mg/L the mortality of these embryos was 75%. At a concentration of 1500 mg/L, it showed 100% lethality within 48 h. This way, it is possible to notice that the extract of the leaves presented toxicity for the embryos of the species *D. rerio*, however, further studies are needed with other methods in Zebrafish to evaluate the leaf extracts of *M. oleifera* used in the evaluation of biological activity in *B. glabrata*.

CONCLUSION

It was possible to observe that the crude extracts in hexane, dichloromethane, ethyl acetate, chloroform, acetone, ethanol, methanol and butanol of the leaves of the species *Moringa oleifera* showed molluscicidal activity on the species *Biomphalaria glabrata*. Among the aforementioned extracts, the crude extract in dichloromethane and the crude extract in hexane showed better activity on the intermediate host. Despite the concentration not being in accordance with that recommended by the World Health Organization (WHO), these extracts present a possibility as an alternative in population control in the vector of schistosomiasis disease. This way, we suggest the search for pharmacological and pharmacotechnical alternatives in order to reduce these molluscicidal concentrations, adapting to what is recommended by the World Health Organization.

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REFERENCES

Anzano, A.; Ammar, M.; Papaianni, M.; Grausto, L.; Sabbah, M.; Capparelli, R.; Lanzotti, V. ***Moringa oleifera* Lam.: A Phytochemical and pharmalogical overview**. Horticulturrae. 2021; 7 (409).

Barreto, B. L.; Lobo, C. G. **Epidemiological aspects and distribution of cases of Schistomiasis in the Northeast Brazil from to 2017**. Revista Enfermagem Contemporânea. 2021; 10 (1): 111-118.

David, C. R. S.; Angeles, A. Angoluan, R. C.; Santos, J. P. E.; David, E. S.; Dulay, R. M. ***Moringa oleifera* (Malunggay) water Extracts Exhibit Embryo-toxic and Teratogenic Activity in Zebrafish (*Danio rerio*) Embryo Model.** Scholars Research Library. v. 8(7): 163-168, 2016.

Ibrahim, A. M.; Abdalla, A. M. **Impact of *Moringa oleifera* seed aqueous extract on some biological, biochemical, and histological aspects *Biomphalaria alexandrina* snails.** Environmental Science and Pollution Research. v. 24, 2017.

Ibrahim, A. M.; Mohamed, F.; Al-Quraishy, S.; Abdel-Baki, A. S.; Abdel-Tawab, H. **Green synthesis of Cerium oxide/Moringa oleifera seed extract nano-composite and its molluscicidsal activities Biomphalaria alexandrina.** Journal of King Saud University – Science. 2021.

Gomes, K. N. F.; Santos, J. A. A.; Faria, R. X. **Aqueous Extracts: An Alternative in the Control of the Intermediate Host of Schistosomiasis.** 2021- 4(3) OAJBS. ID.000302. DOI: 10.38125/OAJBS.000302

Ma, Z.F.; Ahmad, J.; Zhang, H.; Khan, I.; Muhammad, S. **Evaluation of phytochemical and medicinal properties of Moringa (*Moringa oleifera*) as a potential functional food.** South African Journal of Botany. https://doi.org/10.1016/j.sajb.2018.12.002.

McCullough FS et al. **Molluscicides in schistosomiasis control. Bulletin of the World Health Organization.** 1980; 58(5), 681-689.

Melo, R. C.; Lira, A. B.; Alves, M. F.; Bezerra, C. M.; Lima, L. **O uso de plantas medicinais para doenças parasitárias**. Acta Brasiliensis, 1(1):28-32, 2017.

Milla, P. G.; Rocío, P.; Gema, N. 2021. **Benefícios para a saúde dos usos e aplicações da *Moringa oleifera* em produtos de panificação.** *Plants* 10, no. 2: 318. https://doi.org/10.3390/plants10020318

Ministério da Saúde. Secretaria de Vigilância em Saúde. **Departamento de Vigilância Epidemiológica. Vigilância e controle de moluscos de importância epidemiológica : diretrizes técnicas : Programa de Vigilância e Controle da Esquistossomose (PCE) / Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de Vigilância Epidemiológica**.– 2. ed. – Brasília : Editora do Ministério da Saúde, 2008. 178 p. : il. color. – (Série A. Normas e Manuais Técnicos).

Mtemeli, F. L.; Walter, I.; Tinago, T.; Shoko, R. **An assessment of the molluscicidal potencial of *Cucurbita maxima*** seed extracts on *Biomphalaria pfeifferi* and *Bulinus globosus* snails. All Life. 2021, 14 (1), 244-255. DOI: 10.1080/26895293.2021.1901788.

Nmandi, U. C.; Chuka, E. P.; Ann, O. N. M.; U. U. C.; Chukwudi, U. K.; okwudiri, I. M. **Molluscicidal Assessment of Aqueous Extract of *Moringa oleifera* Lam Seed on *Bulinus* Snail for the Control of Schistosomiasis.** South Asian Journal of Research in Microbiology. v. 7(1): 30-39, 2020.

Organização Mundial da Saúde. **Schistosomiasis**. 2022. Disponível em: https://www.who.int/news-room/fact-sheets/detail/schistosomiasis. Acesso em outubro de 2022.

Padayachee, B.; Baijnath, H. **An updated comprehensive review of the medicinal, phytochemical and pharmacological properties of *Moringa oleifera***. South African Journal of Botany. 129 (2020) 304-316.

Ribeiro, I. A. T. A.; Sá, J. L.; Lima, M. V.; Veras, S. T. S.; Aguiar, J. C. R. O. F.; Aires, J. L. F.; Albuquerque, M. C. P. A.; Silva, M. V.; Melo, A. M. M. A.; Navarro, D. M. A. F.; Correia, M. T. S. **Toxic effect of *Croton rudolphianus* leaf essential oil against *Biomphalaria glabrata*, *Schistosoma mansoni* cercariae and *Artemia salina*.** Acta Tropica. 2021, 223.

Rocha-Filho, C. A. A.; Albuquerque, L. P.; Silva, L. R. S.; Silva, P. C. B.; Coelho, L. C. B. B.; Navarro, D. M. A. F.; Albuquerque, M. C. P. A.; Melo, A. M. M. A.; Napoleão, T. H.; Pontual, E. V.; Paiva, P. M. G. **Assessment of toxicity of *Moringa oleifera* flower extract to *Biomphalaria glabrata*, *Schistosoma mansoni* and *Artemia salina*.** Chemosphere. v. 132, 2015.

Saleh, H. A.; Kabary, H. A.; Zayed, K. M. **Assessment of Bacillus aerius and Bacillus toyonensis extracts as biocontrol agents against Biomphalaria alexandrina snails.** Aquaculture Research. 53: 5382-5397, 2022.

Santos, J. A. A.; Cavalcante, V. P.; Rangel, L. S.; Leite, J. C. V. A.; Faria, R. X. **A new technique using low volumes: A new technique to assess the molluscicidal activity using low volumes.** Evidence-Based Complementary and Alternative Medicine. 2017; 10p

Sharma, K.; Kumar, M.; Waghmare, R.; Suhag, R.; Gupta, O. P.; Lorenzo, J. M.; Prakash, S.; Rais, N.; Sampathrajan, V.; Thappa, C.; Anitha, T.; Sayed, A. A. S.; Abdel-Wahab, B. A.; Senapathy, M.; Pandisilvam, R.; Dey, A.; Dhumal, S.; Amarowicz, R.; Kennedy, J. F. **Moringa (*Moringa oleifera* Lam.) polysaccharides: Extraction, characterization, bioactivities, and industrial application.** International Journal of Biological Macromolecules. 2022.

Silva, C. L. P. A. C.; Vargas, T. S.; Baptista, D. F. **Molluscicidal activity of *Moringa oleifera* on *Biomphalaria glabrata*: integrated dynamics to the control of the snail host of *Schistosoma mansoni*.** Revista Brasileira de Farmacognosia. v. 23 (5), 2013.

Souza, D. G. S.; Souza, R. L. T.; Mesquita, D. R.; Álvares, C. M. O. G.; Barbosa, M. P.; Silva, C. L. M.; Leal, A. R. S.; Cardoso, K. T. S. N.; Filho, P. S. P. S.; Silva, E. L. **Desafios e perspectivas do diagnóstico da esquistossomose mansônica no Brasil: revisão de literatura**. Revista Eletrônica Acervo Saúde. 2021, 13 (3), 1-9.

Souza, S. A.; Pereira, A. P. M.; Rosa, P. V. S.; Lages, M. G. G.; Silva, F. N. Alencar, L. K. B.; Laureiro, M. A. B.; Ferreira, M. C.; Castro, G. L. C.; Araújo Neto, A. P. A.; Everton, G. O. **Molluscicidal Activity Against *Biomphalaria Glabrata* Of The Bioactive Nanoemulsion Of The Essencial Oilk Of *Thymus Vulgaris* Linn (Thyme)**. Research, Society And Dvelopment, V. 11 (15), E427111536784, 2022.