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Research Article

Molluscicidal activity of *Nigella sativa* seed, *Azadirachta indica* leaf and *Khaya senegalensis* bark ethanolic extracts against *Bulinus truncatus* snails

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Abstract: In order to control schistosomiasis, a strategy involves eliminating the intermediate host responsible for its transmission. Plant-derived molluscicides have been extensively researched as a cost-effective approach to combat this disease, seeking alternative solutions to synthetic molluscicides. The study aimed to analyze the phytoconstituents and assess the molluscicidal impact of ethanolic extracts on adult *Bulinus truncatus* snails. Dried plant materials were ground into powder, and then extracted using ethanol 96%. Preliminary phytochemical screening was carried out using standard procedures. *In vitro* molluscicidal activity was evaluated for the three plants extracts in different concentrations (125, 250, 500 and 1000 ppm). Every 5 adult snails were placed in a plastic cup containing a specific amount of dechlorinated water with plant sample. Control negative was prepared by placing the snails in dechlorinated water only, the numbers of survived and dead snails were recorded after 24 and 48 hours. The Lethal dose and Standard deviation were calculated. The phytochemical screening revealed the presence of different chemical constituents in the three plants samples. The result showed that all the plants extracts possessed molluscicidal activity against *B. truncatus* snails. The *A. indica* leaf extract was the most effective with LD_{50} of 74.32544 ppm. From the findings of this study, Molluscicide of plant origin could be useful against the common snail species in Sudan; therefore, the selected plants can play a big role in community based schistosomiasis control.

1. INTRODUCTION

Schistosomiasis, also known as Bilharzia, is a health condition often linked to poverty that results in prolonged illness. This disease is contracted when individuals come into contact with freshwater contaminated by the larval stages (cercariae) of parasitic blood flukes known as Schistosomes (Thétiot-Laurent *et al*., 2013). The adult worms, too small to be visible to the naked eye, inhabit the veins that drain the urinary tract and intestines. Globally, Schistosomiasis

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impacts nearly 240 million individuals, with over 700 million people residing in areas where the disease is prevalent.

This infection is most prevalent in tropical and sub-tropical regions, particularly among impoverished communities that lack access to clean drinking water and proper sanitation (Karunamoorthi *et al*., 2018). Urogenital schistosomiasis is caused by *Schistosoma haematobium*, while intestinal schistosomiasis is attributed to *S. guineensis*, *S. intercalatum*, *S. mansoni*, *S. japonicum*, and *S. mekongi*. Schistosomiasis significantly impacts global health, leading to severe morbidity in millions of individuals worldwide (Sulieman *et al*., 2017). Present in Sudan since ancient times, schistosomiasis has become widespread, endemic, and a major public health concern in most Sudanese states (Amin & Abubaker, 2017).

During recent years, the rise in immigration and travel from regions with a high risk of schistosomiasis has resulted in an uptick in imported cases of the disease (Mohamed, 2012). The initial instance of the disease in Sudan was documented by Balfour, who discovered that approximately 17% of children attending a primary school in Khartoum were afflicted with urinary schistosomiasis (Hajissa *et al*., 2019). Currently, schistosomiasis is prevalent throughout all states in Sudan. Its distribution and prevalence have been increasing due to the ongoing expansion of water resource development and the growing movement of the population (Ahmed, 2006; Satti et al., 2022; Mamoun et al., 2016). Since ancient times, people have looked to nature for remedies to treat their illnesses. The use of medicinal plants initially started at an instinctual level, as there was limited knowledge about the causes of diseases and which plants could be used as treatments. Everything was based on experiential knowledge (Ndubisi & Ikechukwu Anthony, 2021; Elbasheir *et al*., 2020). It is estimated that over 50% of current drugs have originated from plants in some capacity. In rural areas, plant-based molluscicides provide an environmentally friendly solution for disease control (Ke *et al*., 2019; Mtemeli *et al*., 2021).

Therefore, the use of plant molluscicides for snail control continues to be one of the preferred methods for managing schistosomiasis. The social, economic, and health impacts of schistosomiasis should not be underestimated (Rinaldo *et al*., 2021). The productivity of rural residents is significantly reduced as they suffer from weakness and lethargy caused by the disease. The high cost and environmental consequences associated with current molluscicides have led to the idea of exploring plants for their natural molluscicidal properties (WHO,1994). This approach aims to find a more affordable and sustainable alternative to synthetic products, making molluscicidal agents more accessible to impoverished communities for treating their water sources. Plant-based molluscicides are environmentally friendly and can be utilized by individuals in rural areas to combat diseases (Qian & Zhou, 2021). *Nigella sativa* L.belongs to the family Ranunculaceae. *N. sativa* seeds have medicinal properties that make them effective in treating worms and skin eruptions. The oil derived from these seeds is also used externally as an antiseptic and local anesthetic (Islam *et al*., 2019). The volatile oil of *N. sativa* showed the highest activity level against both miracidia and cercariae (Abo-Zeid & Shohayeb, 2015). *Azadirachta indica* A.Juss. belongs to family Meliaceae. *A. indica* was found to have molluscicidal effect against *B. pfeifferi*. Crude extract from the plant caused the death of the snails at different concentrations (Mwonga *et al*., 2015). *Khaya senegalensis* (Desr.) A. Juss. belongs to family Meliaceae, it has been traditionally employed in ethnomedicine to address a range of health issues, including rheumatoid arthritis, diarrhoea, cough, emetic, emmenagogue, anti-malarial, jaundice, microbial infections, autoimmune inflammatory diseases, and cancer treatment (Adamu *et al*., 2022). The present work reports the phytochemical composition and molluscicidal activity of ethanolic extracts of *N. sativa*, *A. indica* and *K. senegalensis* on *B. truncatus* snails.

2. MATERIAL and METHODS

2.1. Preparation and Extraction of Plant materials

Nigella sativa seeds were obtained from a local market in Khartoum state, *A. indica* leaves were harvested from the University of Medical Sciences and technology yard and *K. senegalensis* bark was collected directly from the field from western Sudan. The three samples were taxonomically identified and authenticated at the herbarium of the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum -Sudan. The three samples were cleaned, dried, and then they were ground into coarse powder. About 100g of each sample were weighed and extracted with 96% ethanol; the samples were filtered and vacuum concentrated by rotatory evaporator.

2.2. Phytochemical Screening

Preliminary phytochemical screening was performed using standard procedures (Evans, 2002), the various extracts were tested for alkaloids, flavonoids, sterols, triterpenes, tannins, saponins, cardiac glycosides, coumarins, anthraquinones, reducing sugars and lignin's.

2.3. Snails Collection and Preparation

A total of 250 adult snails of the species *B. truncatus* were collected from Alfityhab area in East Nile District, Khartoum-Sudan, using a clean bucket with natural water from the snails' habitat. The snails were kept in a wide bucket filled with dechlorinated water in a cool place ready for examination. An experimental *in vitro* study was conducted at the Laboratory of Pharmacognosy Department, Faculty of Pharmacy, University of Medical Sciences and Technology.

2.4. Bioassay

The molluscicidal efficacy test was carried out according to the method described by Ayi *et al* (2019) with some modifications. Every 5 adult snails of the same size were introduced in a plastic cup containing different concentrations of each plant extract (125, 250, 500 and 1000 ppm). Each experiment was repeated in triplicates. Control negative was prepared by placing the snails in 50 ml dechlorinated water only. Snails were exposed to the extracts at room temperature, and then the number of survived and dead snails was recorded after 24 and 48 hours. Death of the snails was determined by lack of movement and discoloration of the shell. After 24 hours and 48 hours, the percentage of dead snails and LD_{50} values for each extract were calculated , mortality was recorded and all values were expressed as Mean ± SD.

3. RESULTS

3.1. Quantity of Extracts

Different plant samples were extracted with ethanol 96% and percentage yield was calculated. The highest extractive yield was given by *K. senegalensis* bark (16.98%) followed by *N. sativa* (12.3%) while *A. indica* leaves showed the lowest extractive yield (4.19%).

3.2. Qualitative Phytochemical Screening of Plant Extracts

Phytochemical screening of the crude plant extracts of *N. sativa*, *K. senegalensis* and *A. indica* revealed differences in the constituents. *N. sativa* seeds ethanolic extract was found to contain alkaloids, Flavonoids, triterpenes, tannins, Anthraquinones and carbohydrates, while Sterols, saponins, coumarins, cardiac glycosides, reducing sugars and lignin were not detected. On the other hand, the ethanolic extract of the bark of *K. senegalensis* contained alkaloids, triterpenes, tannins, saponins, cardiac glycosides and reducing sugars, but flavonoids, sterols and anthraquinones were not detected. The leaf extract of *A. indica* revealed presence of sterols, tannins and glycosides only while the other compounds were not detected. The results are showed in Table 1.

Table 1. Phytochemical screening of *Nigella sativa*, *Azadirachta indica* and *Khaya senegalensis* extracts.

Test	Sample		
	N.sativa	*K. senegalensis*	*A. indica*
Alkaloids	+ve	+ve	-ve
Flavonoids	+ve	+ve	-ve
Sterols	-ve	-ve	+ve
Triterpenes	+ve	+ve	-ve
Tannins	+ve	+ve	+ve
Saponins	-ve	+ve	-ve
Coumarins	-ve	+ve	-ve
Cardiac Glycosides	-ve	-ve	-ve
Reducing sugars	-ve	+ve	-ve
Anthraquinones	+ve	-ve	-ve
Lignin	-ve	-ve	-ve
Carbohydrates	-ve	+ve	-ve

*Key: (+ve) Detected; (-ve) Not detected

3.3. Molluscicidal Activity of The Crude Extracts

The impact of the tested extracts on *B. truncatus* snails was assessed by tallying the deceased snails. All crude ethanolic extracts displayed a significantly increased in snail mortality at various concentrations. The findings indicated that mortality is dependent on time; furthermore, higher concentrations of the extract led to a significant rise in the mortality rate.

3.3.1. *Nigella sativa*

Molluscicidal activity of *N. sativa* seed extract is presented in Table 2 and Figure 1. The concentrations 1000 ppm and 500 ppm showed a high mortality percentage of 66.67% ± 0.00% in day 1 and 100% in day 2 with an average mortality of 83.33% ± 23.57%.

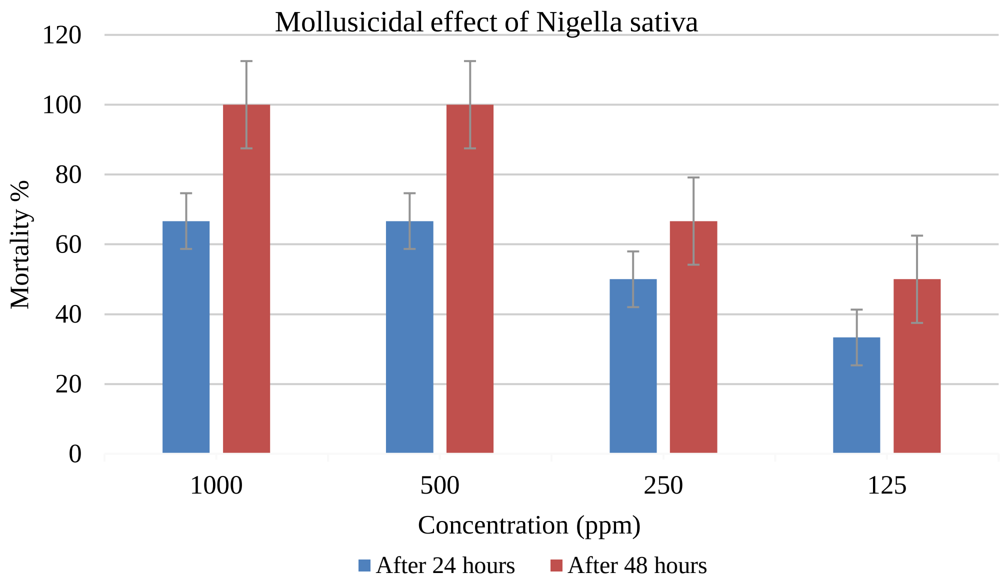

Figure 1. Mortality percentage of *Nigella sativa* seed extract.

Table 2. Molluscicidal effect of *Nigella sativa* seeds extract on *Bulinus truncatus* snails.

Concentration (ppm)	Mortality %		
	After 24 hours	After 48 hours	Mortality average%
1000	66.67±0.00	100±0.00	83.33±23.57
500	66.67±0.00	100±0.00	83.33±23.57
250	50±23.57	66.667±0.00	58.33±11.78
125	33.33±0.00	50±23.57	41.66±11.78
Control -ve (Water)	0 ±0.00	0±0.00	0 ±0.00

3.3.2. *Azadirachta indica*

A. indica leaf extract showed higher toxicity in day 2 with complete death of snails at concentrations 1000 and 500 ppm. The concentration 1000 ppm showed the highest mortality percentage of 91.67 %±11.78%, followed by 500 ppm and 250 ppm with 75%±11.78%, while the lowest concentration (125 ppm) gave the lowest average mortality percentage. The result is shown in Table 3 and Figure 2.

Table 3. Molluscicidal effect of *Azadirachta indica* leaf extract on *Bulinus truncatus* snails.

Concentration (ppm)	Mortality %		
	After 24 hours	After 48 hours	Mortality average%
1000	83.33±23.57	100±0.00	91.67±11.78
500	66.67±0.00	100±0.00	75±11.78
250	66.667±47.14	83.333±23.57	75±11.78
125	50±70.71	66.66±47.14	58.33±11.78
Control -ve (Water)	0 ±0.00	0±000	0 ±0.00

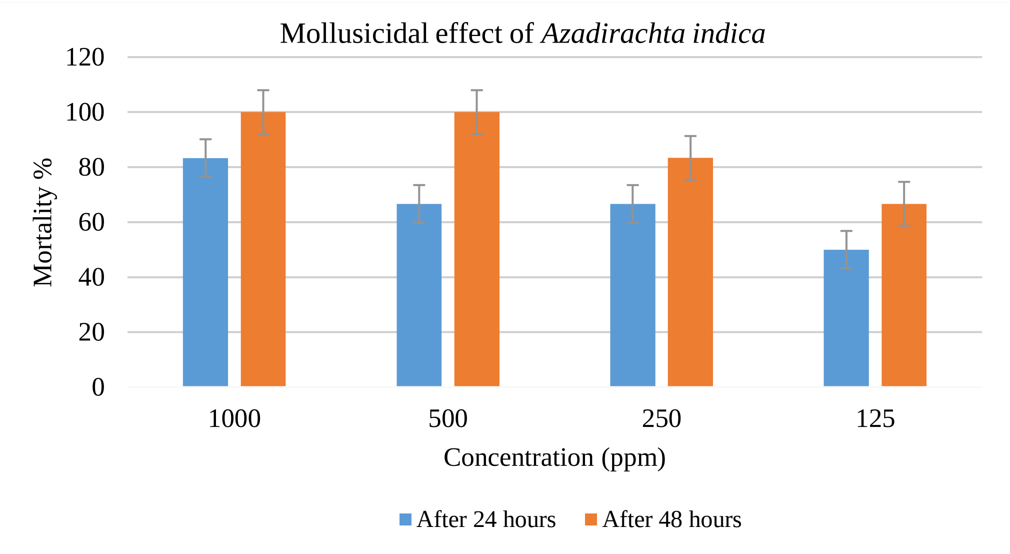

Figure 2. Mortality percentage of *Azadirachta indica* leaf extract.

3.3.3. *Khaya senegalensis*

The *K.senegalensis* achieved the highest mortality level between the three extracts. The results showed in Table 4 and Figure 3. The mortality percentage of the snails was found to be 94.44% ± 7.85% at concentration 1000 ppm, followed by 83.3% ± 7.85 at 500 and 250 ppm respectively.

Table 4. Molluscicidal effect of *Khaya senegalensis* bark extract.

Concentration (ppm)	Mortality %		
	After 24 hours	After 48 hours	Mortality average%
1000	88.89±19.24	100±0.00	94.44±7.85
500	77.78±19.24	88.89±19.24	83.333±7.85
250	77.78±19.24	88.89±19.24	83.33±7.85
125	44.44±19.24	55.56±19.24	50±7.85
Control -ve (Water)	0 ±0.00	0±0.00	0 ±0.00

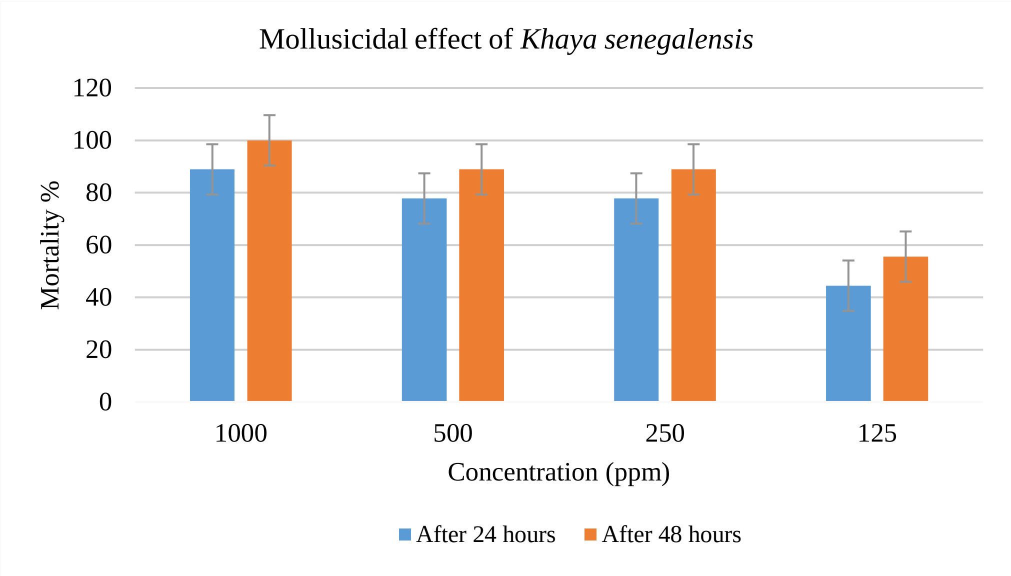

Figure 3. Mortality percentage of Khaya senegalensis bark extract.

3.3.4. *Determination of lethality dose*

LD50 of the extracts was determined to measure the dose that killed 50% of the target organisms within the treatment period. From the present results in this study, the concentrations of plant extracts needed to kill 50%, 90% and 99% of snails were calculated. Among the extracts used on adult snails, *A. indica* leaf extract was the most effective and highly toxic with LD50 of 74.32 ppm. It confirmed that low concentrations were needed to achieve significant death of the snails and that is what makes the extract suitable for use as molluscicide. The results are presented in Table 5.

Table 5. Lethality doses of *Nigella sativa*, *Azadirachta indica* and *Khaya senegalensis* extracts.

Extract	Lethal Doses (ppm)		
	LD_{50}	LD_{90}	LD_{99}
N. sativa	176.77	933.10	1356.60
A. indica	74.32	901.25	1580.10
K. senegalensis	111.36	587.77	854.60

4. DISCUSSION and CONCLUSION

Schistosomiasis continues to pose a significant threat to populations in various parts of the world, particularly in developing countries, where it persists as a major health issue (Verjee, 2019). The search for plants with molluscicidal properties has been intensified, as shown by extensive screening and the overall improved methods and techniques (Barua *et al*., 2021). The importance of understanding the mode of action of plant molluscicides has been stressed by various investigations (Barua *et al*., 2021), in hope that less toxic, cheaper, available, that could be used in control of snail intermediate host. In this study, the screening of *N. sativa* seed extract, *A. indica* leaf extract and *K. senegalensis* bark extract for molluscicidal potency was carried out. The results showed that all extracts possessed molluscicidal properties against the *B. truncatus* snails. Ethanolic leaf extract of *A. indica* had a stronger molluscicidal activity compared to the other two extracts. This could be attributed to secondary metabolites detected in the phytochemical screening of the extract such as; sterols, tannins and cardiac glycosides. Secondary metabolites in plants are responsible for exhibiting molluscicidal action. Previous researches on plants with molluscicidal properties has focused primarily on two significant secondary metabolites; tannins and saponins. These compounds were considered to be the main toxic substances affecting snails. The exact mechanism of tannins' effect is still not fully understood, but it is believed that they can bind with digestive enzymes and proteins. Eventually, after hydrolysis, tannins can transform into toxic substances within the digestive tract (El-Seedi *et al*., 2022).

On the other hand, saponins cause cell lysis in mollusks, leading to the release of lymph and ultimately resulting in their demise (Quintero Santos *et al*., 2022; Piyasena & Qader, 2022). *K. senegalensis* molluscicidal activity may be due to the presence of flavonoids and terpenoids that were reported in other studies to correlate with the molluscicidal activity (Noorshilawati *et al*., 2020). In addition to the effect of the tannins and saponins that was previously explained. In a previous study, several bioactive phytochemical constituents that have specific physiological effects have been documented, including alkaloids, flavonoids, tannins, terpenoids, saponins, and phenolic compounds (Nigam *et al*., 2020). Most of these phytochemicals were found in the selected plants. The target site of saponins includes muscle, hemolymph, intestine and hepatopancreas (stomach poison) of freshwater snails. Other previous studies have indicated that flavonoids and saponins can hinder the breathing process, potentially by impeding the diffusion of oxygen through the gills of golden apple snails. This obstruction is believed to be caused by the secretion of mucus (Abdullah *et al*., 2017). In another study, alkaloids are highly toxic against vectors of *Schistosomiasis*(Ke *et al*., 2017); cardiac glycosides also proved to have good molluscicidal effect (Dai *et al*., 2011; Kashyap *et al*., 2019).

Utilizing the potential of medicinal plants as natural molluscicides presents a promising alternative to synthetic chemical agents, offering environmentally friendly solutions for controlling mollusk populations and mitigating their adverse effects on human activities. From the observations in the present study, the three plants possessed molluscicidal activity against *B. truncatus* adult snails. Hence, it is the most suitable option for biological applications, providing a potentially simple, readily available, inexpensive, and environmentally safe molluscicidal agent of plant origin for controlling human schistosomiasis in Sudan. Plant-based molluscicides could serve as alternatives to costly synthetic products, enhancing the accessibility of treatment for water collections in impoverished communities. Further research is needed to extract and identify the active components of these plant extracts.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Rawan Osama Abdelaziz: Performed sample collection, Laboratory work and performed analysis. **Ayat Ahmed Alrasheid**: Initiate the idea, participated in analysis, Supervision and wrote the original draft. **Ahmed Saeed Kabbashi**: performed the laboratory work and participated in the analysis. **Gokhan Zengin**: Critical revision and final approval of the article. **Saad Mohammed Hussein Ayoub**: Responsible for conceptualization and investigation of the study, consultation and review the article. All authors have read and approved the final manuscript.

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REFERENCES

Abdullah, N.S., Aziz, N.A., Mailon, R. (2017). Molluscicidal activity of *Entada rheedii* stem bark methanolic extract against paddy pest *Pomacea canaliculata* (Golden Apple Snail). *Malaysian Journal of Analytical Sciences, 21*(1), 46-5. http://dx.doi.org/10.17576/mjas-2017-2101-06

Abo-Zeid, K., Shohayeb, M. (2015). Evaluation of the biocidal activity of alkaloids, saponins and volatile oil extracted from *Nigella sativa* seeds against miracidia and cercariae of *Schistosoma mansoni*. *International Journal of Pharmaceutical Science Invention, 4*(1), 47-54.

Adamu, I., Majiyebo, A.J., Alozieuwa, U.B., Lawal, A.A., Alawode, B.O.O.R.A., & Berinyuy, E.B (2022). Ethnopharmacology, chemical constituents, and biological activities of *Khaya senegalensis* (Desr.) A. Juss a forest plant with multi-purpose applications. *AROC in Natural Products Research*, *2*(2), 10-15. https://doi.org/10.53858/arocnpr02021015

Ahmed, A.A. (2006). Schistosomiasis in sugar cane schemes, Sudan. *Sudan J Nat Sci*, *4*, 1-11. Amin, M., & Abubaker, H. (2017). Control of schistosomiasis in the Gezira irrigation scheme, Sudan. *Journal of Biosocial Science, 49*(1), 83-98. https://doi.org/10.1017/s0021932016000079

Ayi, Fabrice Chandre, I., Coelho, P.M.Z., El-Harawy, A.K., Elemam, M.A., *et al*. (2019), *Guidelines for laboratory and field testing of molluscicides for control of schistosomiasis*. Rajpal Singh Yadav. World Health Organization, 978 92 4 151540 5. hal-02378870. https://hal.science/hal-02378870

Barua, A., Williams, C.D., & Ross, J.L. (2021). A literature review of biological and bio-rational control strategies for slugs: current research and future prospects. *Insects*, *12*(6), 541. https://doi.org/10.3390/insects12060541

Dai, L., Wang, W., Dong, X., Hu, R., Nan, X. (2011). Molluscicidal activity of cardiac glycosides from *Nerium indicum* against *Pomacea canaliculata* and its implications for the mechanisms of toxicity. *Environmental Toxicology and Pharmacology, 32*(2), 226-32. https://doi.org/10.1016/j.etap.2011.05.007

Elbasheir, M.M., Karti, I.A., & Elamin, E.M. (2020). Evaluation of a rapid diagnostic test for *Schistosoma mansoni* infection based on the detection of circulating cathodic antigen in urine in Central Sudan. *PLoS Neglected Tropical Diseases*, *14*(6), e0008313. https://doi.org/10.1371/journal.pntd.0008313

El-Seedi, H.R., Khalifa, S.A., Mohamed, A.H., Yosri, N., Zhao, C., El-Wakeil, N., & Verpoorte, R. (2022). Plant extracts and compounds for combating schistosomiasis. *Phytochemistry Reviews*, 1-116. https://doi.org/10.1007/s11101-022-09836-x

Esquilla, M.G., Sanchez, C.B., Tenorio, J.C. (2021). Molluscicidal activity of Makabuhay (Tinospora rumphii Boerl) stem ethanolic extract against *Radix* (Lymnaea) spp. Snails. *Journal on New Biological Reports*, *10*(2), 64-71.

Evans, W.C. (2009). *Trease and evans Pharmacognosy*. 9th Edition published by Saunders Elsevier. 553.

Hajissa, K., Muhajir, A.E., Eshag, H.A., Alfadel, A., Nahied, E., Dahab, R., ... & Mohamed, Z. (2018). Prevalence of schistosomiasis and associated risk factors among school children in Um-Asher Area, Khartoum, Sudan. *BMC Research Notes*, *11*(1), 1-5. https://doi.org/10.1186/s13104-018-3871-y

Islam, M.T., Khan, M.R., & Mishra, S.K. (2019). An updated literature-based review: Phytochemistry, pharmacology and therapeutic promises of *Nigella sativa* L. *Oriental Pharmacy and Experimental Medicine*, *19*, 115-129. https://doi.org/10.1007/s13596-019-00363-3

Kanu Ikechukwu, K., Ndubisi, E.J.O., & Nwadialor, K.L. (2021). African Religion And Culture Honoring the Past and shaping the future. First Edition. Editors: Kanu, I.A., Nwadialor, K.L., Ndubisi, E.J.O., *Innovative trends in African traditional medicine* (pp. 45-64). Association for the Promotion of African Studies, Maryland.

Karunamoorthi, K., Almalki, M.J., & Ghailan, K.Y. (2018). Schistosomiasis: A neglected tropical disease of poverty: A call for intersectoral mitigation strategies for better health. *Journal of Health Research and Reviews*, *5*(1), 1-12. https://doi.org/10.4103/jhrr.jhrr_92_17

Kashyap, S., Khagta, S., Guleria, K., & Arya, V. (2019). Plants as Molluscicides: A recent update. *International Journal of Botany Studies*, *4*(1), 50-56.

Ke, W., Lin, X., Yu, Z., Sun, Q., Zhang, Q. (2017). Molluscicidal activity and physiological toxicity of *Macleaya cordata* alkaloids components on snail *Oncomelania hupensis*. *Pesticide Biochemistry and Physiology*, *143*, 111-115. https://pubmed.ncbi.nlm.nih.gov/29183578/

Ke, W., Tu, C., Cao, D., Lin, X., Sun, Q., & Zhang, Q. (2019). Molluscicidal activity and physiological toxicity of quaternary benzo [c] phenanthridine alkaloids (QBAs) from *Macleaya cordata* fruits on *Oncomelania hupensis*. *PLoS Neglected Tropical Diseases*, *13*(10), e0007740. https://doi.org/10.1371/journal.pntd.0007740

Mutamad, A., Mohamed, K., Noma, M., Hwiada, A., Algali, M., & Mamoun, H. (2016). The Transmission Patterns of Shistosomiasis in Khartoum State, Sudan. *Annals of Clinical Pathology*, *4*(6), 1088.

Mohamed, Issam A.W. (February 27, 2012). Water Borne Diseases and Rural Development Study of Bilharzias in Gezira Irrigated Agricultural Scheme, Sudan Available at SSRN: http://dx.doi.org/10.2139/ssrn.2011943

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

Mwonga, K.B, Waniki, N.E, Dorcas Y.S, Piero, N.M (2015). Molluscicidal effects of aqueous extracts of selected medicinal plants from Makueni County, Kenya. *Pharm Anal Acta.*, *6*(11), 445-59. https://doi.org/10.4172/2153-2435.1000445

Nigam, M., Mishra, A.P., Adhikari‐Devkota, A., Dirar, AI., Hassan, MM, Adhikari, A., Belwal T., Devkota, H.P. (2020). Fruits of *Terminalia chebula* Retz.: A review on traditional uses, bioactive chemical constituents and pharmacological activities. *Phytotherapy Research*, *34*(10), 2518-2533. https://doi.org/10.1002/ptr.6702

Noorshilawati, A.A., Suraya, A., & Rossiyah, S.S. (2020). Molluscicidal activity *of Ipomoea batatas* leaf extracts against Pomacea canaliculata (Golden apple snail). *Food Research*, *4*(5), 131-137. https://doi.org/10.26656/fr.2017

Piyasena, K.G.N.P., & Qader, M.M. (2022). *Chemistry of Natural Products: Phytochemistry and Pharmacognosy of Medicinal Plants*. Berlin, Boston: De Gruyter, 2022. https://doi.org/10.1515/9783110595949

Qian, Y., & Zhou, X. (2021). *Schistosomiasis Risk and Prevention*. *In Prevention and Control of Infectious Diseases in BRI Countries*, Springer Singapore, 143-156. https://www.infontd.org/resource/schistosomiasis-risk-and-prevention

Quintero Santos, C.D., Castellanos, L., & Becerra-Rozo, W.M. (2022). Possibilities of the alternative management of mollusk pests in agricultural crops. A review. *INGE CUC*, *18*(1), 1-13. http://doi.org/10.17981/ingecuc.18.1.2022.01

Rinaldo, D., Perez-Saez, J., Vounatsou, P., Utzinger, J., & Arcand, J.L. (2021). The economic impact of schistosomiasis. *Infectious Diseases of Poverty*, *10*(1), 1-12. https://doi.org/10.1186/s40249-021-00919-z

Satti, A.B., Mohammed, W.M., Garbi, M.I., & Hamad, M.N.M. (2022). Prevalence of Schistosoma Haematobium Infection in Alsyal Village, River Nile State, Sudan. *SAR Journal of Pathology and Microbiology*, *3*(6), 81-84. https://doi.org/10.36346/sarjpm.2022.v03i06.001

Schenkel, E.P., Gosmann, G., Athayde, M.L. (2007). Saponinas. In: Farmacognosia: da planta ao medicamento. 6ª Porto Alegre: Editora da UFRGS; Florianópolis: Editora da UFSC, 711–740.

Sulieman, Y., Eltayeb, R., Pengsakul, T., Afifi, A., Zakaria, M., & Khairala, M. (2017). Schistosomiasis as a disease and its prevalence in Sudan: An overview. *Journal of Coastal Life Medicine*, *5*(3), 129-133. https://doi.org/10.12980/jclm.5.2017J6-247

Thétiot‐Laurent, S.A.L., Boissier, J., Robert, A., & Meunier, B. (2013). Schistosomiasis chemotherapy. *Angewandte Chemie International Edition*, *52*(31), 7936-7956. https://doi.org/10.1002/anie.201208390

Verjee, M.A. (2019). Schistosomiasis: still a cause of significant morbidity and mortality. *Research and Reports in Tropical Medicine*, *10*, 153-163. https://doi.org/10.2147/RRTM.S204345

World Health Organization. (1994). Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: report of a WHO study group [meeting held in Rome from 22 to 25 June 1992].