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Evaluation of the effectiveness of *Saponaria officinalis* plant on the infection of *Biomphalaria alexandrina* snails with *Schistosoma mansoni*

Menna M. Fathy, Nada W. Younis, Omnia T. Ahmed, Reham TH. Mohamed, Salma E. Ahmed, Sara M. Mohamed, Sohaila A. Hussein

Supervisor: Rania G. Taha, Assistant Professor, Parasitology, Department of Biological and Geological Science, Faculty of Education, Ain Shams University.

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Abstract

Schistosomiasis is one of the most prevalent tropical diseases worldwide and the second most common parasite in the world after malaria in terms of the number of cases and endemic areas. As resistance to praziquantel, the recommended medication as natural products of plants and easily biodegradable for treating schistosomiasis, grows, more sophisticated treatments are being looked for. Saponaria officinalis contains a rich array of bioactive compounds, including saponins, flavonoids, and phenolic acids, which have demonstrated various pharmacological properties. The stem of the plant was dried and grinded to prepare five different concentrations with three replicates for each and testing against "*Biomphalaria alexandrina*" snails. Results showed that the lethal concentration that led to the mortality of all snails was 175 ppm of the while LC10, LC25, LC50 and LC90 were 67.43, 88.05, 110.95 and 154.46 ppm respectively. The study offers plenty of opportunity to use native resources in the area to find molluscicidal agents for harmful snails.

Key Words:

Schistosoma mansoni- *Saponaria officinalis*- *Biomphalaria alexandrina*- Schistosomiasis- Molluscicide

1. Introduction:

Schistosomiasis, also known as snail fever, is a parasitic disease caused by trematode worms of the Phylum: *Platyhelminthes* (Flatworms), Class: *Trematoda* (Flukes), genus *Schistosoma*. (WHO, 2023). It is one of the most prevalent tropical diseases, affecting over 200 million people worldwide as *Schistosoma haematobium* (urinary blood fluke) is found in Africa and the Middle East., *S. mansion* is found in Africa, Egypt (Nile Delta) Middle East (CDCP, 2023). *Schistosoma japonicum* is found in the Far East, Japan, Philippines, China, and Indonesia. The disease is transmitted through contact with contaminated freshwater, where the parasite's larval stage, cercariae, penetrates the skin. Once inside the human body, the parasite matures and migrates to its target organ, typically the liver, intestines, or bladder. *Schistosoma* can cause a range of histopathological effects, including liver, urinary tract, and lung damage. The eggs of the parasite are deposited in these organs, causing inflammation and scarring. This can lead to various problems, such as liver cirrhosis, nodules in the bladder, and pulmonary hypertension (Walker et al. 2021). Current treatment for schistosomiasis relies on praziquantel, a medication that effectively kills adult *schistosomes*. The development of safe, effective, and sustainable treatment options is crucial for combating this disease. However, praziquantel does not eliminate the parasite's eggs, leading to reinfection in endemic areas. Additionally, concerns about drug resistance have emerged, prompting the search for alternative and more sustainable treatment strategies. (WHO, 2023).

Saponaria officinalis (Fm. Rosaceae) is a promising natural remedy commonly known as soapwort. It is a flowering plant with a long history of medicinal use in traditional herbal remedies. It contains a rich array of bioactive compounds, including saponins, flavonoids, and phenolic acids, which have demonstrated various pharmacological properties (Lin & Harnack, 2013). On the other hand, it has antiparasitic properties and potential to modulate the host's immune response, holds promise as a novel therapeutic agent for schistosomiasis. Further research and clinical evaluation are essential to establish its efficacy and safety in human populations. In recent years, Saponin shows significant miracidicidal and cercaricidal activity against the free-swimming larval stages of *Schistosoma mansoni*. This disrupts the parasite's lifecycle within the snail host (Ibrahim et al. 2023). Other research concerning the effect of plant extracts on the surviving and feeding of *B. alexandrina* snails such as (El-Sherbini et al. 2009) who used the solvent extract of fresh mature leaves of *Solanum* sp. Certain kinds of snails are linked to the intermediate host role in the spread of parasitic diseases. Biological control appears to be a more effective strategy than chemical controls used to suppress snail populations. One of the top research priorities for scientists working on the control of alternative molluscicides is the search for herbal formulations that are readily biodegradable and do not have any negative effects on non-target creatures so that the aim of our study is to evaluate the molluscicidal activity of the dried stem of plant *saponaria officinalis* against *Biomphalaria alexandrina* as a new approach for controlling these snails.

2. Materials and Methods

2.1. Test Snails:

Snails were gathered from various irrigation channels in Abo-Rawash, Giza province. Snails, measuring (9 to 12 mm diameter) of genus *Biomphalaria alexandrina* (Ehrenberg, 1831) were kept in the Medical Malacology Laboratory at Theodor Bilharz Research Institute (TBRI) in Giza, Egypt. The snails were housed in 16 x 23 x 9 cm plastic aquariums containing dechlorinated tap water and fed with Tetramin, blue-green algae (Nostoc muscorum), and oven-dried lettuce leaves. The aquaria were filled with dechlorinated aerated tap water (10 snails/L), with a pH of 7±0.2, a temperature of 25±2 C^o, and glass plates covering the water. For snail fertility, growth, and shell length, 30 mg/L of calcium carbonate, or $CaCO_3$, was used to optimise water hardness (Eveland and Haseeb, 2011).

Fig. (1) ***Biomphalaria alexandrina*** **(Ehrenberg, 1813)**

2.2. Plant material:

The plant of the present study was selected according to its pharmacological properties and antiparasitic activity against *B. alexandrina* and *Schistosoma mansoni*. It was obtained from Faculty of Agriculture, Cairo University, Giza, Egypt. The stem of plants was grinded, powdered and kept for use as molluscicides.

Fig. (2) The plant *S. officinalis* (Fm. Rosaceae) fresh and powder

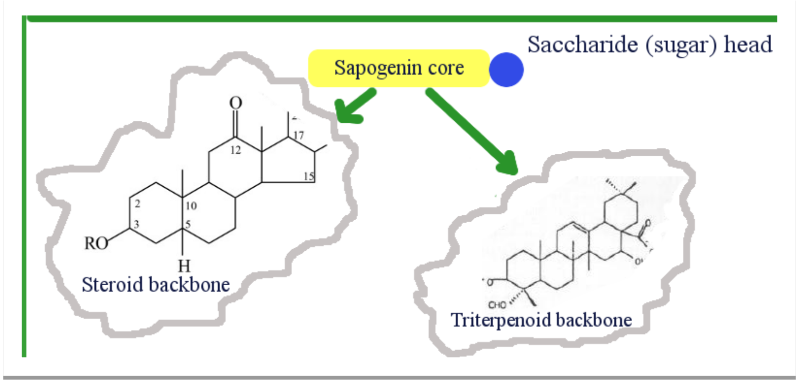

Fig. (3) showed The chemical structure of Saponin material cited from: https://www.medicinalplants-pharmacognosy.com/pharmacognosy-s-topics/glycosides/saponins/

2.3 Experimental design

Ninety-five juvenile and adult snails (9 to 12 mm) were divided into 7 groups, the first group with five snails was control and the rest of groups (5 snails in each group with three replicates) were exposed to different concentrations of dried plant material dissolved in dechlorinated tapwater as following (50-75-100-125-150-175 ppm) for 24 hours and then snails were transferred to new aquaria for recovery.

Fig. (4) Different test solutions of plant material, ranging from 50 to 175 mg/L, (i.e, *ppm*) were prepared using dechlorinated tap water, to determine the different LC values.

2.4 Total Phenolic Compounds (TPC):

Using the Folin-Ciocalteu reagent and the method outlined by Singleton and Rossi (1965), the total phenolic compounds of sample were determined spectrophotometrically. The blue-coloured complex was quantified at 765 nm (UV-1800, Shimadzu, Kyoto, Japan). A calibration curve for gallic acid (10-160 µg/mL) was employed. The findings were reported as grammes of gallic acid equivalents (GAE) (g of GAE/100 g) for each 100 g of sample. The temperature and RH% of the environment during the analysis were 27 and 40% respectively.

Results

Total Phenolic Compounds (TPC):

The total phenols which were determined in the present study was 1.04 g/100g of stem powder.

Molluscicidal activity of *Saponaria officinalis* extract against survival rate of *Biomphalaria alexandrina* snails:

It has been showed that the survival rate of the snails was affected by the exposure to different concentrations of plant extract. The lethal concentration of plant extract which led to the death of all snails was 175 *ppm*. While the concentration at which all snails were still alive was 50 *ppm* **(Table, 1)**

Extract con. *ppm*	Dead snails	%	Live snails	%
50	**0**	**0**	**15**	**100**
75	**3**	**20**	**12**	**80**
100	**6**	**40**	**9**	**60**
125	**10**	**67**	**5**	**33**
150	**12**	**80**	**3**	**20**
175	**15**	**100**	**0**	**0**

Table (1) shows the survival rate of *B. alexandrina* snails due to the effect of different concentrations of the plant extract *S. officinalis* after 24 hours of exposure.

Confidence Limits				
	Probability	95% Confidence Limits for conc		
		Estimate	Lower Bound	Upper Bound
PROBIT	.010	31.962	-11.377	54.404
	.050	55.101	22.905	72.474
	.100	67.437	40.908	82.380
	.250	88.049	70.030	99.892
	.500	110.950	99.001	122.735
	.900	154.463	139.748	180.436

Table (2) shows the different LC values calculated by the methods of Leitchfield and Wilcoxon (1949) at 95% confidence limit.

In the present work, the values of LC_{10}, LC_{25}, LC_{50} and LC_{90} of plant extract *S. officinalis* for snails were calculated using SPSS program at 95% confidence limit, they were 67.43, 88.05, 110.95 and 154.46 *ppm* respectively **(Table,2).**

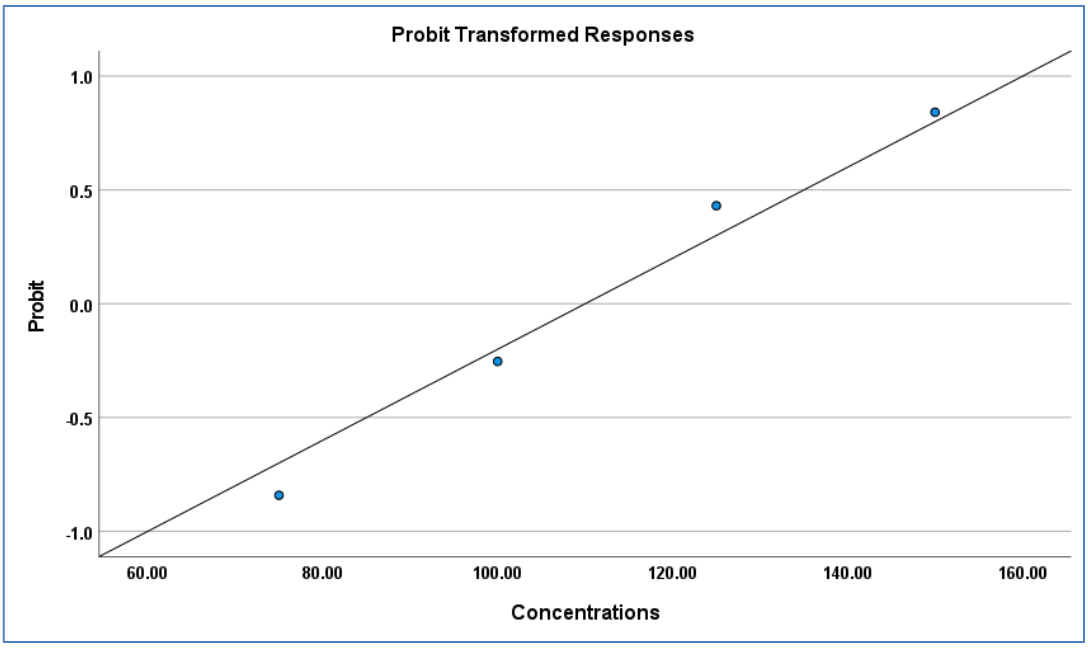

Fig. (5) shows the effect of different LC values of the tested material on the tested snails.

Discussion

Schistosomiasis continues to occupy the second position in the world among parasitic diseases, after malaria (Moustafa & Soliman, 2010). A vast array of illnesses and disorders have been treated with natural compounds derived from plants and herbs, serving as substitute medications. Because of the unfavourable side effects and prohibitively high cost or restricted efficacy of the antibiotics, chemicals, or medications now in use (Harvey et al. 2015). Higher plants include a large variety of glycosides called saponins. These substances differ from other glycosides due to their surface-active characteristics. They dissolve in water to create colloidal solutions, which shake to produce foam (Reichert et al. 1986). Because some saponins serve as the foundation for the semi-synthesis of steroidal medicines, they have also been sought after by the pharmaceutical sector. Numerous possess pharmacological characteristics and find application in phytotherapy and the cosmetic sector (Sparg et al. 2004).

Two kinds of saponins can be distinguished according to the makeup of their aglycone skeletons. The steroidal saponins, which are virtually exclusively found in monocotyledonous angiosperms, make up the first category. The most prevalent type, the triterpenoid saponins, are found mostly in dicotyledonous angiosperms (Bruneton, 1995).

A surprising number of saponins are poisonous so they are called sapotoxins, if injected straight into a vein they will haemolyze red blood cells. The primary characteristic of these plant sterol-related active substances is their abundance of hydroxyl groups as well as ether and lactonic type connections. Saponins contain the water-soluble material oily glycosides which cause foaming when they are agitated. They have been employed in place of soap in several detergent applications, particularly for foaming purposes, primarily in fire extinguishing liquids (https://www.medicinalplants-pharmacognosy.com/pharmacognosy stopics/glycosides/saponins/). There are kinds of snails that act as intermediary hosts in the spread of parasitic diseases. If chemical controls are used to prevent snails, biological control is probably a preferable option. One of the key study topics for scientists working on the control of alternative molluscicides is the search for herbal formulations that are readily biodegradable and do not negatively affect non-target creatures. (El-Sherbini et al. 2009).

In the present work the lethal concentration of dried solvent plant *S. officinalis* is 175 *ppm* which led to the mortality of all snails and this concentration is higher than (90 *ppm*) recorded by (El-Sherbini et al. 2009) for the solvent extract of fresh mature leaves of *Solanum* species against *B. alexandrina* snail. However, the values of LC_{50} and LC_{90} in the present work are 110.95 and 154.46 *ppm* respectively and these results are higher than 69.9 & 96.5 *ppm* that introduced by (Mandefro et al. 2017) during assess the molluscicidal effect of the aqueous extract *Achyranthes aspera* against *Biomphalaria* sp. Also, it is higher than 20 & 120 *ppm* recorded by (Motowe, 1993) for using the effect of gypsogenin from *Saponaria officinalis.* The reason for the difference in results between the mentioned studies is the type species of plant used in each study, which can lead to differences in concentrations and LC values. The composition and properties of each plant vary, affecting the efficacy of their extracts. (Moreover, Abououf et al. 2018) evaluated the molluscicidal activity of the methanol extract of plants *Anagallis arvensis* and *Viburnum tinus* against *B. alexandrina* and proved a high activity of both species at LC_{50} & LC_{90} (45- 60 *ppm*) and (38- 59 *ppm*) respectively. (Motowe, 1993) demonstrated that after five days of continuous exposure to a 500 *ppm* saponin solution from *S. officinalis*, 100% of the specimens died. (Pereira et al. 2014) investigated the effects of fruit, leaf, and stem extracts of *Jatropha gossypiifolia* on *B. glabarata*. They recorded 100% mortality of all snails at 25*ppm* and considered that stem extract didn't exhibit molluscicidal activity.

The present results also agree with that introduced by (Ibrahim et al. 2023) as they proved that saponin exhibits potent molluscicidal activity against *Biomphalaria alexandrina* (LC50 was 70.05 mg/l) and they also showed that it has Antimiracidial and Anticercacidial activity. Schistosomiasis incidence and prevalence were significantly reduced by the use of molluscicides in snail management (Abououf et al. 2018).

The total phenols which were determined in the present study was 1.04 g/100g of plant powder and this value is higher than (0.81 g/100g) reported by (Sengul et al. 2011) from the same species, this difference may be due to the treatment of plant after harvesting, genetic characters, environmental conditions (Coronel et al. 2022). Future research on the best growing, drying, storing, and extracting conditions for *S. officinalis* bioactive components will be required to benefit these characteristics in this plant species locally. The saponin material that occurred in different plants have a toxic effect against slugs. They stated that the applying plant extracts high in saponins may be a suitable way to manage slugs because food and agronomic goods in the US, EU, and Japan have shown to be safe when containing saponin residues. Additionally, they pose no risk to the environment because they don't hurt plants or the farmer who uses them (Cruz & San

Martin, 2013). The lethal effect of saponin substance as observed in the present study may be due to its harmful effect on the digestive gland of the mollusk or its impacts on the nervous system as suggested by (Zaldibar et al. 2007). *Saponaria officinalis* dried plant demonstrated significant molluscicidal activity against *Biomphalaria alexandrina* snails, the intermediate host of *Schistosoma mansoni*. This could be owed to its high content of Triterpenic saponins. There is an immediate need for new, environmentally safe, and naturally based control techniques.

Conclusion

Schistosomiasis continues to pose a serious threat to public health, especially in areas with little resources. The creation of sustainable, safe, and effective treatment alternatives is essential to the fight against this illness. With its proven antiparasitic qualities and ability to alter the host's immunological response, *Saponaria officinalis* has great potential as a new therapy for schistosomiasis. Its safety and efficacy in human populations must be established by more study and clinical assessment.

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