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Original Article

Molluscicidal Activity of Acacia seyal (Dell) Bark Methanolic Extract Against *Biomphalaria pfeifferi* Snails

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The *Acacia seyal* Dell family Mimosaceaeis is known locally as Talha tree. Many medicinal uses such as treatment of dysentery, leprosy, colds, diarrhea, hemorrhage, ophthalmic, intestinal ailments, rheumatic pains, colds and fevers, have been attributed to this tree. Schistosomiasis, a water-borne parasitic disease caused by Schistosome(s) a genus of digenetic trematode worms, infects man and animals in tropical and sub tropical regions. It is the second most prevalent parasitic disease in the world ranked only after malaria regarding the number of people infected and those at risk. More than 90% of the cases are occurring in Africa. The purpose of this study was to investigate the molluscicidal activiat ty of *Acacia seyal* bark methanolic extract against the snail *Biomphlaria pfeiffer*. Different concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125 and 3.91 ppm) of the *Acacia seyal* bark methanolic extract, were investigated *in vitro* against snail *Biomphlaria Pfeiffer*. *A. seyal* bark methanolic extract exhibited 100% mortality within 24 h, only at a concentration of 1000 ppm; and exhibited 100% mortality within 48 h, at 1000, 500, 250, 125 and 62.5 ppm. The IC_{50} of bark of *A. seyal* at 24 h and 48 h was 80.79 and 34.33 ppm, respectively. These findings prove the potent *in vitro* activity of *A. seyal* (bark) methanolic extract against *Biomphlaria pfeiffer*.

Keywords: Molluscicidal activity, *Acacia seyal* (bark), *Biomphlaria* snails, *in vitro*, Sudan

In Sudan, Shistosomiasis is considered to be one of the major health problems which seem to spread in parallel with the expansion of agricultural irrigation strategies and tools which create a suitable habitat for snails as intermediate hosts for the disease (1). Poor communities are much threatened

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by this disease, because health resources are most limited and people lack access to safe drinking water and adequate sanitation (2). The causative agents of the disease are the blood trematodes of the genus Schistosoma. These parasites undergo part of their development in fresh water snails, which serve as intermediate hosts (3). There are at least 350 snail species with medical or veterinary importance. However, those serving as hosts for human Schistosoma parasites mostly belong to three genera, Biomphalaria, Bulinus, and Oncomelania.

Schistosomiasis is a disease of various mammals, including man and domestic livestock, caused by blood flukes from the genus Schistosoma (4). It is endemic in 74 countries. It is wide spread in Africa, the Middle East, the northern part of South America and South East Asia. The World Health Organization (WHO) estimated that approximately 250 million people are suffering from the infection and that more than 600 million people reside in areas where schistosomiasis is transmitted (5).

Various factors are responsible for the increasing importance of schistosomiasis. The most significant factors are the expanding use of fresh water in the tropics to meet the increase in demands for food production. The construction of irrigation system and dams for water storage and electrical power production has created new and highly suitable habitats for the fresh water snails, which play a vital role in the transmission of schistosomiasis. Other factors include lack of sufficiently trained health workers, adequate safe water, improper facilities, high cost of chemicals for snail control and the therapeutic drugs (6).

Due to the high cost and environmental effect of molluscicides currently in use for the control of schistosomiasis, finding new sources of molluscicides, especially those of plant origin is of particular interst (7). Plant molluscicides can prove to be an ideal source of low cost, safe and effective molluscicides (8-9).

Today, natural products derived from plants are being tested for the presence of new drugs with new modes of pharmacological action. A special characteristic of higher plants is their capacity in producing a variety of secondary metabolites (10). Recent studies aimed to identify and isolate new therapeutic compounds from higher plants for diseases treatment or prevention (11-12). *Acacia seyal* Genus is Acacia Mill.–acacia Family Fabaceae–Pea family Species Acacia seyal Delile–talh. Acacia have been used in chewing sticks as an antimicrobial agent against Streptococcus facials. It was also shown to have some cholesterol lowering and antidiabetic properties. Acacia is used in medicinal emulsions and troches, helping to mask the effect of bitter substances. Acacia extract presents both aphrodisiac and cytotoxic effects (13). It is used in traditional medicine to treat bleeding, lymphoid fever diarrhea, and many infections including those affecting the upper respiratory tract (14).

Acacia seyal (Mimosaceae) is known locally in Sudan as (Talih) a small to medium-sized tree, growing up to 17 m tall (15). It has thin red brown bark. The inflorescence is a bright yellow, axillary pendunculate, globose head. Pods are slightly curved, thin, and 7-20 cm long (16). *Acacia seyal* is native to Sudan, Egypt, Eritrea, Ethiopia, Ghana, Iran, Kenya, Malawi, Mali, Mozambique, Namibia, Niger, Nigeria, Saudi Arabia, Senegal, Syrian Arab Republic, Tanzania, Uganda, Yemen, Republic of Zambia, and Zimbabwe. Moreover, it is exotic to Afghanistan, Bangladesh, Bhutan, India, Nepal, Portugal, Sri Lanka and the US (15).

Acacia seyal may be used for food as Gum talha, which is eaten in fresh form either alone or toguether with local foods. The bark is extensively used for feeding farm mammals during the dry season. The pods and leaves are nutritious and

palatable to livestock. Moreover, *A. seyal* var. seyal is an important source of rural energy as both firewood and charcoal. In Sudan, it is used to make a fragrant fire over which women perfume themselves (16).

Snail control through the use of synthetic molluscicides forms an important part in the integrated schistosomiasis control programme (17). However, the high cost of synthetic compounds such as Bayluscide® (Bayer, Leverkusen, Bayerwerk, Germany) and their negative impact on the environment have stimulated interest in search for alternative molluscicides of plant origin (18). Many plant species have been proved to have molluscicidal properties against different snail species. The most potent plant that has been known to have molluscicidal activity against snail intermediate hosts of schistosome is *Phytolacca dodecandra* (19). Other plants such as *Alternanthera sesselis* (20), *Balanites aegyptiaca* (21) and *Jatropha curcas* (22) have also shown molluscicidal activities. Plant molluscicides may be produced locally and serve as a source of cheap, biodegradable and efficient agent in countries with endemic schistosomiasis. The present study was carried out to evaluate the *in vitro* Molluscicidal activity of the methanol extract of *Acacia seyal* bark against *B. pfeifferi.*

Materials and methods

Plant materials

The bark *A. seyal* was collected from central Sudan between January 2014 and February 2014. The plant was identified and authenticated by the taxonomists of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI) Khartoum, Sudan. Bark of *A. seyal* was air-dried under the shade, and pulverized and stored prior to extraction. The shading was accompanied with good ventilation and then plants were ground finely in a mill and kept in the herbarium until their uses for extracts preparation.

Preparation of crude extracts

Extraction was carried out according to the method described in Harbone (**1984**). To prepare extracts for screening of molluscicidal activity, bark was separated from the plant. Specific weight of each sample (about 50 g of the powdered sample) was successively extracted with methanol (80%) using soxhlet extractor apparatus. Extraction was carried out for about 6 h for methanol (80%) using soxhlet extractor apparatus, or 6-8 h for methanol solvents more evaporated under reduced pressure using rotary evaporator. Extracts were allowed to air dry. Finally, the extracts were freed from solvent with the aid of rotary evaporator and the percentage yield was calculated.

Parasite collection

The parasite was collected using a scoop from the Major canals and the Mean canals of "Abuashrin" and Minor canals of “El Seilait” agricultural schemes in Khartoum state and Abuashar, “El taklajobara” agricultural schemes in Aljazeera state. A series of laboratory experiment were designed and conduced in indoor aquaria at the department of microbiology, faculty of pure and applied science, International University of Africa, in May 2015.

Molluscicidal activity assay

The molluscicidal potency tests against mature adult (5-7 mm) snails were conducted in accordance with WHO (23) guidelines. *Biomphlaria pfeifferi* snails, the intermediate host of *S. mansoni* snail, were collected using a scoop. Pilot experiments were conducted to detect the toxicity range of *A. seyal* (bark) samples extract. Three replications of serial concentrations (1000, 500, 250 and 125 ppm) of the plant samples extract were performed in the pilot experiment. 3 snails were put in each beaker containing either 50 ml of different concentrations

Table 1. Inhibition concentration (IC) of *A. seyal* (bark) methanol extract against *B. Pfeifferi*

IC (µg/ml)	Time/days	
	After one day	After two days
IC_{50}	80.79	34.33
IC_{90}	512.46	162.29
IC_{95}	645.58	197.07
IC_{99}	776.57	230.19

IC_{50}: inhibition concentration 50%; IC_{90}: inhibition concentration 90%; IC_{95}: inhibition concentration 95%; IC_{99}: inhibition concentration 99%.

of *A. seyal* methanolic extract or 50 ml of distilled water as a control. Molluscicidal activity examination for the extracts were performed after 24 h and 48 h of exposure.

According to the results of the pilot experiments, the most effective plant sample was selected with regard of 100% average mortality within 48 h period of time. The most effective plants samples were subjected to additional investigation using concentrations (62.5, 31.25, 15.625, 7.8, and 3.9 ppm) through the same steps. The mortality of the snail was detected by its motionlessness or discoloration of the shell. Dead snails were counted and then percentage mortality and percentage average mortality were calculated for each concentration.

Each test was replicated two times after 24 h and 48 h from the assay. The number of dead snails was expressed as % mortality.

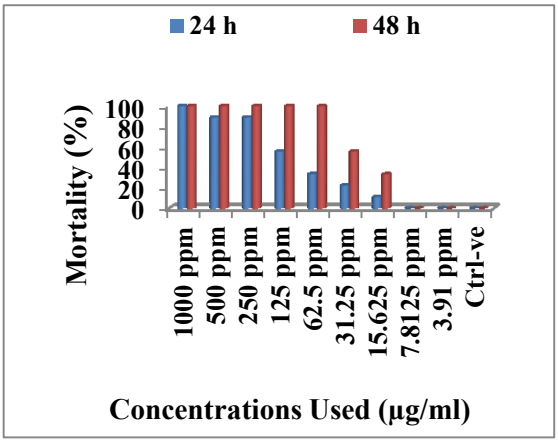

Figure 1. The activity of *Acacia seyal* bark methanol extract aginst *B.Pfeifferi*.

Statistical analysis

All data were presented as means ± S.D. Student T test was used to compare the mean differences between control and extracts used against snail. The level of significance was used for $P < 0.05$. The microsoft Excel (2007) program was used to calculate the inhibition concentration IC_{50}, IC_{90}, IC_{95} and IC_{99} values by applying linear regression equations.

Results

The methanolic extract of *A. seyal* (bark), with different concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125 and 3.91 ppm) were investigated against snail *Biomphlaria pfeiffer*. *A. seyal* (bark) methanolic extract exhibited 100% mortality within 24 h, at a concentration of 1000 ppm; and exhibited 100% mortality within 48 h, at concentrations of (1000, 500, 250, 125 and 62.5 ppm) (Figure 1). The 24 h and 48 h IC_{50} of *A.* seyal methanolic extract were 80.79 and 34.33 ppm, respectively (Table 1).

Discussion

Mollusciciding is still considered as the most important mean of controlling schistosomiasis transmission. In rural communities the cost and environmental consequences of synthetic molluscicides limit their use. Plant molluscicides,

may be obtained as crude extracts and serve as cheap and biodegradable effective alternative agents. The discovery of presence of molluscicidal agents in some plants like *Phytolacca dodecandra* (Phytolacaeae) from Ethiopia has led to a large amount of investigations on plant-derived compounds showing toxicity against schistosomiasis-transmitting snails (8, 19).

The results of the tested plant extract, screened against *B. pfeifferi* (Table 1 and figure 1), were obtained based on methods used for determining plant efficacy, according to standards required for the screening of molluscicide (23) and by determining the toxicity of methanolic extracts of *A. seyal* (bark) against snails of the genus *B. pfeifferi* which is a vector of the intestinal form of schistosomiasis.

The Molluscicidal activity of many Sudanese medicinal plants against a variety of snail vectors of tropical diseases have been well investigated (24-30). Most of the extracts from these medicinal plants show remarkable molluscicidal activity against snails *Biomphalaria Pfeifferi* and *Bulinus truncatus*.

These results which indicate anti-parasitic properties agree with traditional uses of *A. seyal* in Sudan. The plant extracts were found to exhibit antibacterial (31), antimicrobial (32), and antioxidant activities during phytochemical screening (33).

This is the first evaluation of this plant against snails. The obtained results suggest the possibility of using an alternative tool for the control of schistosomiasis. Bioassay-directed fractionation of the active crude materials, may lead to the isolation and identification of the compound(s) responsible of the molluscicidal activity, and to understand their mechanisms of action.

From the present study, it can be concluded that the *Acacia seyal* (bark) methanolic extract has molluscicidal properties and can be used as a molluscicide in the control of schistosomiasis. This make the present experiment another addition to experiments carried out to investigate the use of plant products in controlling the Schistosoma snail intermediate host so as to prevent schistosomiasis infection.

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Conflict of interest

The authors declared no conflict of interest.

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