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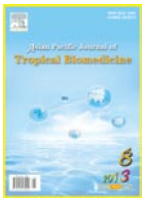

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Laboratory assessment of the molluscicidal and cercariacidal activities of *Balanites aegyptiaca*

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PEER REVIEW

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Comments

This is a useful study which is adequately organized and written. The preliminary findings of the molluscicidal and cercariacidal properties of the crude extract of *B. aegyptiaca* have demonstrated encouraging results for further studies.
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ABSTRACT

Objective: To assess the molluscicidal and cercariacidal activities of aqueous extracts of *Balanites aegyptiaca* (*B. aegyptiaca*) against Ethiopian *Biomphalaria pfeifferi* (*B. pfeifferi*), *Lymnaea natalensis* (*L. natalensis*) and *Schistosoma mansoni* (*S. mansoni*) cercariae.
Methods: Extracts of seeds, endocarp, mesocarp, and fruit of *B. aegyptiaca* were tested for their activities against adult *B. pfeifferi* and *L. natalensis*. The cercariacidal activity of the seeds of the plant was also evaluated against *S. mansoni*. Bioassays were carried out following the methods recommended by WHO. Snail mortalities were compared between each plant part and snail species, and LC_{50} and LC_{90} values for the plant parts tested were computed. The cercariacidal activity of the plant was assessed by exposing the mice to the cercariae pre-exposed to aqueous extract of *B. aegyptiaca* seeds.
Results: For the molluscicidal activities of seeds, endocarp, mesocarp and whole fruit, the LC_{50} values against *B. pfeifferi* were 56.32, 77.53, 65.51 and 66.63 mg/L, respectively, while the respective LC_{90} values were 77.70, 120.04, 89.50 and 97.55 mg/L. Similarly, the LC_{50} values for the seeds, endocarp, mesocarp and whole fruit against *L. natalensis* were 80.33, 92.61, 83.52 and 87.84 mg/L, respectively, while the respective LC_{90} values were 102.30, 138.21, 115.42 and 127.69 mg/L. *B. pfeifferi* were found to be more susceptible to *B. aegyptiaca* than *L. natalensis*. *S. mansoni* cercariae exposed to 15 mg/L of extract of seeds were incapable of infecting mice. The mean egg load of tissue was reduced in mice infected with the cercariae exposed to 5 and 10 mg/L of the extract.
Conclusions: The aqueous extracts of different parts of *B. aegyptiaca* exhibited reasonable molluscicidal activity against *B. pfeifferi* and *L. natalensis*, as well as cercariacidal activity against *S. mansoni* cercariae. However, comprehensive laboratory evaluation is recommended prior to field tests of the plant parts since their impact on other aquatic biota is not known.

KEYWORDS
Balanites aegyptiaca, Molluscicide, *Biomphalaria pfeifferi*, *Lymnaea natalensis*, Cercariacide, *Schistosoma mansoni*

1. Introduction

Schistosomiasis is one of the most prevalent parasitic worm infections and has significant economic and public health consequences. It affects many countries, particularly in sub-Saharan Africa[1]. It is estimated that over 200 million people are infected with *Schistosoma* and over 600 million people are reported to be at risk[2,3]. The five most common species of schistosome infecting humans are *Schistosoma mansoni (S. mansoni)*, *Schistosoma japonicum*, *Schistosoma haematobium*, *Schistosoma mekongi*, and *Schistosoma intercalatum*[4]. The snails of the genera *Biomphalaria*, *Bulinus* and *Oncomelania* serve as intermediate hosts of schistosomes

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and play a crucial role in the transmission of the disease[5].

Fascioliasis is an important helminthic infection caused by trematodes of the genus *Fasciola*. The distributions of etiologic agents overlap in many areas of Africa and Asia[6]. Fascioliasis is one of the most important parasitic diseases in tropical and subtropical countries[7]. The World Health Organization (WHO) has estimated that 2.4 million people are infected with *Fasciola* and 180 million are at risk of infection[8].

The high cost of synthetic molluscicides and their negative impacts on the environment, as well as fear for emergence of snail resistance to these compounds have given a new impetus to the study of molluscicidal plants. Several plants, such as *Phytolacca dodecandra* (Endod), *Solanum xanthocarpum*, *Annona squamosa*, *Thuja orientalis*, *Stryphnodendron polyphyllum*, *Calotropis procera* and *Adenium arabicum* have already been identified as useful to control the intermediate hosts of trematodes[9-14].

Balanites aegyptiaca (*B. aegyptiaca*) is a tree, classified as a member of the family Balanitaceae. *B. aegyptiaca*, deep rooted arid zone tree has a very wide natural range. The tree is valued for its fruits and seeds. The seed kernel is rich in oil, protein, minerals and is edible as snacks after boiling. The wide range of habitat in which this species is occurring suggests high pattern of variation among and within locations[15].

Various parts of *B. aegyptiaca* tree have been used for folk medicines in many regions of Africa and Asia[16]. Its fruit, bark and other parts have lethal properties to snail intermediate hosts, schistosome miracidia and cercariae, and the cercariae of other trematodes[17]. Nevertheless, the molluscicidal and cercariacidal properties of the plant have not been studied in Ethiopia. The purpose of the present study, therefore, was to evaluate the molluscicidal and cercariacidal activity of aqueous extracts of *B. aegyptiaca* seeds and fruits in Ethiopia.

2. Materials and methods

2.1. Collection and preparation of the plant materials

The seeds and fruits of *B. aegyptiaca* were collected from Ziway and Arsi Negele areas, in the Oromia Regional State of Ethiopia. The plant was authenticated by a botanist, and voucher specimens were deposited at the National Herbarium of Addis Ababa University, (specimen No. Eshetu 001). Mature unripe *B. aegyptiaca* fruits (Figure 1) were washed with tap water. The epicarp (outer cover) of the fruits was removed by using sterile sharp surgical blade, and the fruits pulps/mesocarps were scraped manually and the seeds were then collected. The endocarp of the fruits was broken manually and the seeds were then collected. The prepared fruit parts (endocarp and mesocarp), the whole fruit and seeds were air dried under shade in the laboratory for 5-6 d, separately[16]. Thereafter, the dried specimens were manually ground into powder using mortar and pestle. The powder, then, was sieved through 250 microns mesh to obtain a fine material. The powdered fine plant material was then transferred into closed containers until use.

Figure 1. *B. aegyptiaca*, a branch with fruits.

2.2. Collection of the snails

The snails *Biomphalaria pfeifferi* (*B. pfeifferi*) and *Lymnaea natalensis* (*L. natalensis*) were collected from different areas. *B. pfeifferi* were collected from Chacha, Senbete and Mekele (Northern Ethiopia). *L. natalensis* were collected from Wondo Genet (Southern Ethiopia) and Jiga (Northwest Ethiopia). The snails were then transported to the laboratory at Aklilu Lemma Institute of Pathobiology, Addis Ababa University for maintenance. They were then left to acclimatize to standard laboratory conditions before being used in the experiments. Snails were maintained in the aquaria containing dechlorinated tap water at room temperature[7].

2.3. Preparation of the stock solution and serial dilution

The concentrations used in the bioassays were prepared from fruits (endocarp and mesocarp), seeds and the whole fruit in successive dilutions with aged water. One gram of each powdered dry plant part used in the tests was weighed using an electric balance. The weighed powdered dry part was soaked in 1 000 mL of aged water for 24 h with occasional vigorous shaking using a shaker. Then, the suspension was filtered using filter paper (Whatman No. 1).

After the preparation of the stock solution of 1 000 mg/L, successive concentrations of the aqueous extracts were prepared to obtain the final concentrations of 65, 70, 75, 80, 85, 90, 95, 100 mg/L for testing against *B. pfeifferi* snails. And for *L. natalensis* snails, 85, 90, 95, 100, 105,

110, 115 and 120 mg/L, in a final volume of 500 mL in each solution were prepared. For each test, there was a control with aged water, without plant material, with the same volume of the solution[10,18].

2.4. Molluscicidal activity tests

Water extracts of *B. aegyptiaca* seeds, endocarp, mesocarp, and fruit were tested against adult *B. pfeifferi* and *L. natalensis* snails according to the method recommended by WHO[19]. To each concentration of plant parts used, 10 adult snails of both *B. pfeifferi* and *L. natalensis* were immersed. Each test concentration was set up in duplicate. Tests were carried out at room temperature. After 24 h of exposure, the suspension was decanted, the snails were rinsed thrice with aged water and transferred to another aged water and maintained there for another 24 h recovery period. Ten snails were immersed in separate aged water with the same volumes of the solvent that would serve as control. Two replicates for each test were used. All groups were observed carefully after 24 h, the number and the percentages of death in each group were calculated. Snails were considered dead if they could not move or retracted well into or hanging out of the shell, with the body and shell discolored[20].

2.5. Cercariacidal activity tests

Infected *B. pfeifferi* snails were individually placed in shedding vials containing 5 mL of aged water and exposed to artificial light for 30 min. The emerging cercariae were pooled and counted with the aid of microscope. Three glass beakers containing 500 mL of 5, 10 and 15 mg/L of water extract of *B. aegyptiaca* seeds prepared in aged water were set up in duplicates. Over 300 cercariae were slightly caught with pipettes and transferred to each beaker and allowed to stand for 2 or 4 h. Similarly, six glass beakers containing 500 mL aged water were set up in duplicates. After exposing the cercariae for 2 or 4 h to these concentrations, 200 cercariae (pre-determined infective dose) were again transferred from beaker to another beaker containing aged water. In each of the beakers one mouse was placed and allowed to stay for 40 min for exposure by the paddling method (through skin penetration of the legs). After 40 min of exposure to cercariae the mice were returned to their cages and maintained in the animal house under standard conditions. Control mice were exposed to the same numbers of cercariae that were not exposed to the seeds extract[21].

After 45 d post exposure to the cercariae, the faecal samples of mice were collected and checked for the presence of schistosome eggs. Each infected mice were sacrificed to determine the mean egg load in each tissue (liver, small and large intestines). Tissue egg load was determined by digesting known weights of liver, small intestine and large intestine by mechanical grinder. After grinding each tissue, about 0.10 g of each tissue was observed in microscope and the eggs were counted. The number of eggs obtained were then used to extrapolate for the total weight of each tissue and finally converted to number of eggs per gram of tissue[21]. The mean of numbers of egg loads for the total mice perfused for each concentration was calculated by geometric mean, using the formula:

$$\text{Geometric mean} = \text{Exp}\frac{\Sigma \log (\text{epg} + 1)}{n} - 1$$

Where,

epg=the number of eggs per gram of tissue;

Exp=exponential or antilogarithm;

log (epg + 1)=the sum of the logarithm of each mouse epg;

n=the number of mice perfused in each concentration.

2.6. Data analysis

Probit regression analysis using SPSS program version 13.0 was carried out for all tested parts of the plant to determine the LC_{50} and LC_{90} values against both snail species with 95% confidence intervals (*CI*). Analysis for variance (One-way ANOVA) was used to determine the significant reduction in tissue egg load in the mice.

3. Results

3.1. Molluscicidal activity

The molluscicidal potency of all tested parts of *B. aegyptiaca* against both *B. pfeifferi* and *L. natalensis* were concentration dependent. Generally, mortality increased with the increase in concentration of the extracts.

The lethal concentrations for aqueous extracts of *B. aegyptiaca* seeds, endocarp, mesocarp and the whole fruit that killed 50% (LC_{50}) of adult *B. pfeifferi* were 56.32, 77.53, 65.51 and 66.63 mg/L, respectively, while the respective LC_{90} values were 77.70, 120.04, 89.50 and 97.55 mg/L (Table 1).

Table 1
Effect of aqueous extract of *B. aegyptiaca* on mortality rates of *B. pfeifferi* after 24 h of exposure.

Plant parts	LC_{50} (mg/L)	LC_{50} (*CI*)	LC_{90} (mg/L)	LC_{90} (*CI*)
Seeds	56.32	(36.99-63.61)	77.70	(71.97-86.63)
Endocarp	77.53	(70.15-83.11)	120.04	(104.18-178.95)
Mesocarp	65.51	(56.31-70.35)	89.50	(83.81-102.16)
Fruit	66.63	(55.64-72.09)	97.55	(89.42-120.35)

The lethal concentrations for aqueous extracts of *B. aegyptiaca* seeds, endocarp, mesocarp and the whole fruit that killed 50% (LC_{50}) of adult *L. natalensis* were, 80.33, 92.61, 83.52 and 87.84 mg/L, respectively, while the respective LC_{90} values were 102.30, 138.21, 115.42 and 127.69 mg/L (Table 2). Comparing the LC_{50} and LC_{90} values of the plant parts, seeds showed the highest molluscicidal activity, followed by mesocarp, whole fruit and then the endocarp against both snail species.

Table 2
Effect of aqueous extract of *B. aegyptiaca* on mortality rates of *L. natalensis* after 24 h of exposure.

Plant parts	LC_{50} (mg/L)	LC_{50} (*CI*)	LC_{90} (mg/L)	LC_{90} (*CI*)
Seeds	80.33	(66.38-86.38)	102.30	(97.35-111.92)
Endocarp	92.61	(79.88-98.76)	138.21	(121.97-212.11)
Mesocarp	83.52	(68.15-90.03)	115.42	(107.53-139.06)
Fruit	87.84	(72.93-94.19)	127.69	(115.59-174.56)

3.2. Cercariacidal activity

The infectivity of the cercariae to mice was evaluated by exposing the mice to the cercariae pre-exposed to aqueous extract of *B. aegyptiaca* seeds. The infection was completely inhibited at 15 mg/L, at 2 or 4 hours of exposure of cercariae to the seeds extract. Concentrations lower than 15 mg/L significantly reduced tissue egg load ($P<0.05$) (Table 3).

Table 3
The *in vivo* observation on *S. mansoni* cercarial infectivity of aqueous extract of *B. aegyptiaca* seeds.

Exposure time	Concentration (mg/L)	No. of mice	GM		
			SI	Liver	LI
2 h	Control	2	80.44	52.94	48.00
	5	2	50.40	35.55	39.09
	10	2	37.60	26.70	25.93
	15	2	0.00	0.00	0.00
4 h	Control	2	62.18	59.74	47.46
	5	2	27.46	32.50	29.29
	10	2	9.32	15.82	10.94
	15	2	0.00	0.00	0.00

LI=Large intestine; SI=Small intestine; GM=Geometric mean of egg counts per gram of tissue.

4. Discussion

The present study showed that aqueous extracts of *B. aegyptiaca* seeds and fruits possess molluscicidal and cercariacidal properties. Their activities are time and concentration-dependent. Between the test snail hosts of trematodes, *B. pfeifferi* were more susceptible to the plant extract than *L. natalensis*. Aqueous extracts of *B. aegyptiaca* seeds showed the highest molluscicidal activity followed by mesocarp, whole fruit and endocarp on both test snail species after 24 h of exposure period. The seeds and fruits showed high molluscicidal activity against both snails. Previous investigators also reported similar observations[20].

The varying potencies of each plant part may be due to the differences in concentration and/or the type of the active ingredient(s) present in each part[22]. In this observation, water extracts of seeds and mesocarps were more potent than the water extract of endocarps, perhaps due to the more concentrations of saponins in the mesocarps and seeds, and also presence of high amount of deltonin in the seeds.

The LC_{50} values of aqueous extracts of the seeds and fruit observed against *L. natalensis* in this study were higher than the values reported by Vijay[20]. Vijay obtained LC_{50} values of 60 mg/L after the exposure of *L. acuminata* to aqueous extracts of *B. aegyptiaca* fruits for 24 h. In the present study, the LC_{50} values against *L. natalensis* were 80.33 mg/L for seeds, 92.61 mg/L for endocarp, 83.52 mg/L for mesocarp , and 87.84 mg/L for fruit. On the other hand, the LC_{50} value of the seeds against *B. pfeifferi* was 56.32 mg/L, but for other plant parts the LC_{50} values were above 60 mg/L (77.53 mg/L for endocarp, 65.51 mg/L for mesocarp and 66.63 mg/L for whole fruit). In another study, Anto *et al*. reported that extracts of *B. aegyptiaca* fruits have molluscicidal activity with LC_{95} values of 19.7 mg/L and 12.0 mg/L against adult *Bulinus globosus* and *Bulinus truncatus*, respectively[23].

In Sudan, Ragab *et al*. studied the molluscicidal activity of this plant and found out that *B. aegyptiaca* saponins (seeds) showed significant effect on *Bulinus truncatus* at different concentrations[24]. They also observed that the mortality of snails increased with increasing concentrations and exposure period.

Compared to some other plants tested for their molluscicidal activities, *B. aegyptiaca* has high toxicity to snail hosts of trematodes for some while for others it has low molluscicidal property. The LC_{50} values for *Cymbopogon nervatus* against *B. pfeifferi* are less than 213.099 mg/L[25]. Abdalla *et al*. observed that the LC_{50} and LC_{90} values for ethanolic extract of *Euphorbia aphylla* against *Biomphalaria alexandrina* were 87.6 mg/L and 142.5 mg/L, respectively[26]. Hassan *et al*. also observed that the LC_{50} and LC_{90} values of butanol extracts of *Meryta denhamii* fruits against *Lymnaea natalensis* were 26.4 mg/L and 70.8 mg/L, respectively[27], which is lower than the present study against the same snail species. The LC_{50} and LC_{90} of *Ziziphus spinachristi* ethanolic extract against *L. natalensis* snails were 311 mg/L and 500 mg/L respectively, which is higher as compared to the current study[26].

In order for a plant to be considered as candidate molluscicide, its crude extract should be active at 100 mg/L or lower when 90% of the snails are killed after 24 h exposure[28]. The results of the current observation indicated that aqueous extracts of the seeds, mesocarp and whole fruit caused 90% *B. pfeifferi* mortality after 24 h exposure time at 77.70, 89.50 and 97.55 mg/L, respectively. Hence, this plant could be a potential molluscicide for *B. pfeifferi*. On the other hand, the LC_{90} values for *B. aegyptiaca* against *L. natalensis* were above 100 mg/L, that is, 102.30, 115.42 and 127.69 mg/L for seeds, mesocarps and fruits, respectively, indicating that aqueous extract of this plant parts are less potent against *L. natalensis*.

The present *in vivo* observation on cercariacidal properties of *B. aegyptiaca* showed that water extract of *B. aegyptiaca* seeds made *S. mansoni* cercariae less infective to mice at lower concentrations. *S. mansoni* cercariae exposed to 15 mg/L of aqueous extract of seeds were incapable of infecting the mice. Furthermore, at 5 and 10 mg/L, the mean tissue egg load was significantly reduced. This observation is in agreement with that of others who used different plant species as schistosomicide. Birrie *et al.* showed that pre-treatment of the cercariae with 12 mg/L of the endod berries completely inhibited infection of mice and significantly reduce egg deposition in tissue even below a concentration of 12 mg/L[21]. Abozeid *et al.* also demonstrated that tannins extracted of pomegranate (*Punica granatum*) have a remarkable antischitosomal activity particularly on miracidia[29]. Both molluscicidal and cercariacidal activities of extracts of *Punica granatum* were demonstrated. Rind methanol and water extracts were lethal to 100% of cercariae at concentrations of 25 and 30 mg/L, respectively, after 24 h[29].

From the present study, it can be concluded that aqueous extracts of seeds and fruits of *B. aegyptiaca* have reasonable molluscicidal activity against *B. pfeifferi* and *L. natalensis*, and cercariacidal activity against *S. mansoni* cercariae. The aqueous extracts of *B. aegyptiaca* seeds were more toxic to the cercariae of *S. mansoni* than to *B. pfeifferi*, snail intermediate hosts of *S. mansoni*.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Trematode infection is one of the major problems leading to enormous health and economic losses in Ethiopia. Commercial molluscicidal or cercariacidal drugs are relatively expensive and this is further complicated by the development of drug resistance and toxicity to aquatic life. Screening and proper evaluation of medicinal plants could offer possible alternative for sustainable and affordable use. The results of this preliminary study are interesting and warrant further studies.

Research frontiers

Various studies are currently underway in many developing countries to assess the effects of crude plant products, by applying different extraction techniques and using *in vitro* and *in vivo* techniques. Active ingredients demonstrating promising results separated from plant products should undergo acute and chronic toxicity studies both on the hosts and environment.

Related reports

A number of *in vitro* and *in vivo* studies of medicinal plans are currently underway to examine the antiparasitic and antibiotic properties based on the very promising work on schistosomiasis control using medicinal plant products. Such studies should be encouraged and supported.

Applications

The results of this study can make a significant contribution in the control of fluke infections in humans and animals provided further *in vitro* and *in vivo* studies. Most products having molluscicidal proporties often produce toxic effect on aquatic life. Hence possible undesirable effects of this plant product should be assessed.

Peer review

This is a useful study which is adequately organized and written. The preliminary findings of the molluscicidal and cercariacidal properties of the crude extract of *B. aegyptiaca* have demonstrated encouraging results for further studies.

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