

# Laboratory Assessment of Molluscicidal and Cercaricidal Activities of *Balanites aegyptiaca* against Vectors of Schistosomiasis (*Biomphalaria pfeifferi*)

Muhammad Isa

Department of Microbiology, Yobe State University Damaturu, Nigeria.

## ABSTRACT

Studies were conducted on assessment of molluscicidal and cercaricidal activities of leaves, fruits and endocarp of *Balanite aegyptiaca* Del against adult vectors of schistosomiasis (*Biomphalaria pfeifferi*) using standard methods. Preliminary phytochemical screening was conducted, where alkaloids, saponins, flavonoids were found present in leaves, fruits, and endocarp. Molluscicidal and cercaricidal activity test were also conducted. Snail mortalities were compared between each plant part and snail specie as well as LC<sub>50</sub> of the plant parts were also recorded. The result obtained revealed that leaves extract was more susceptible to the death of the snail species. Comparing LC<sub>50</sub>, the leaves extract shows 0.0726 considered as the highest cercaricidal while fruits and endocarp showed highest LC<sub>50</sub> of 0.0531 and 0.0426 respectively.

**Keywords:** *Balanites Aegyptiaca*, Molluscicidal, Cercaricidal Activities, Schistosomiasis (*Biomphalaria pfeifferi*)

## INTRODUCTION

Schistosomiasis is a disease commonly found in sub-Saharan Africa, and is one of the most prevalent parasitic infections that have significant economic and public health effect. Mbata, (2008) It has been estimated that over 200 million people are infected with *Schistosoma* and over 600 million people are reported to be at risk. (Ibrahim 2009).

According to Mc Manus and Loukas (2008), the five most common species of *Schistosoma* affecting human are *S. mansoni*, *S. japonicum*, *S. haematobium*, *S. mekongi*, and *S. intercalatum*. Snails of the genera; *Biomphalaria*, *Bulinus*, and *Oncomelania* serve as intermediate host of *Schistosoma* and play a vital role in the transmission of the disease.

*Balanite aegyptiaca* is a desert tree classified as a member of the family *Balanitaceae*. It is deep rooted arid zone tree and has a very wide natural range. The tree has been evaluated for its fruits and seeds. The seed kernel is rich in seed and oil as well as protein and minerals and it serve as edible as snacks after boiling. The wide range of it habitat in which these species are found suggests high value of variation among and within locations (Elfeel 2010).

According to Champagain *et al.*, (2005), different parts of this tree is used for folk medicines in many sub Saharan African regions. its leaves, fruits and endocarp have lethal properties to snail intermediate host, schistosome, miracidia and cercariae and the cercaria of other trematodes. Therefore, the purpose of this study is to assess the molluscicidal and cercaricidal activity of *B. aegyptiaca* extract leaves, fruits and endocarp.

## **MATERIALS AND METHODS**

### **Sample Collection and Identification**

The leaves and fruits of *Balanite aegyptiaca* were collected at Geidam Local Government Area in Yobe State, Nigeria with latitude of 11.716120 and longitude of 11.968205, this site was selected because of the availability of riverine area and severe cases of schistosomiasis. The identification and authentication of plant leaves was done in the herbarium of Department of Biological Sciences Yobe State University Damaturu with the following voucher number (YSUHAN 1479) the voucher specimens were deposited at the herbarium for future reference. The leaves and fruits were thoroughly washed with distilled water, following which, leaves were air-dried under shade at room temperature for three weeks, grinded using a wooden pestle and mortar to obtain the powdered material. The powder was kept in a clean sample container and closed until required for use. The fruits were washed and soaked in distilled water overnight, sieved using laboratory sieve (Pore size of 0.05um), freeze-dried under -20 degrees for 30 minutes and later placed in a lyophilizer. The extract was kept at room temperature until further use (Ali *et al.*, 2008)

### **Phytochemical Extraction**

Ethyl acetate, ethanol and distilled water were used in the extraction process. One hundred grams of the powdered leaves were weighed using analytical weighing balance (Drawel H-52) and macerated in 1000ml of ethyl acetate, ethanol and distilled water respectively in a separate clean sample bottle and placed on a laboratory universal shaker (Lead scientific V-2211) for 72 hours. The mixtures were filtered using number 1 Whatman filter paper, the extracts were evaporated to dry in a rotary evaporator.

### **Extract Preparation**

The dried solid powder residue were obtained, measured and reconstituted in 10% DMSO at a stock concentration of

300mg/L. different concentrations were made from the stock solution and preserved at 4°C for further use.

### **Phytochemical Screening**

Preliminary phytochemical screening was carried out in order to determine bioactive metabolites of *Balanite aegyptiaca* leaves and fruits extracts. The presence of saponins, phenols, steroids, flavonoids, cardiac glycoside, tannin and terpenoids were identified using the method described by (Sofowara 2002), Trace; Evans (2002 and 1993).

#### **Test for Alkaloids**

**Mayer's Test:** Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

#### **Test for phenols**

**Ferric Chloride Test:** Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

#### **Test for tannins**

**Gelatin Test:** To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

#### **Test for flavonoids**

**Alkaline Reagent Test:** Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

#### **Test for Saponins**

**Froth Test:** Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

**Test for glycosides:** Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

**Modified Borntrager's Test:** Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

#### **Test for phytosterols**

**Salkowski's Test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

#### **COLLECTION OF SNAILS**

Snails were collected in a perforated plastic container with mud, clay and shrubs enabling conducive environment from Kaleri village in Geidam Local Government Area of Yobe State. They were collected as described by (Yusuf *et al.*, 2005). Samples were transported to Biology research laboratory Yobe State University Damaturu and acclimatized in an aquaria which contained dechlorinated water with spinach for feeding until further usage.

#### **PREPARATION OF STOCK SOLUTION AND SERIAL DILUTION**

The concentration of the stock solution used in the bioassay was prepared from the fruits and leaves, these were diluted with distilled water and 10% DMSO. 1g of the extract was weighed and dissolved in 10ml of 10% DMSO. From the stock, various concentrations of extract were prepared thus 60ug, 65ug, 70ug, 75ug, 80ug, 85ug, 90ug, 95ug, and 100ug/ml of the extracts were prepared.

#### **MOLLUSCICIDAL ACTIVITY TEST**

To each concentration of the extract prepared 10 snails were inoculated and each concentration was prepared in duplicate. Tests were conducted at room temperature at the interval of 6 hours for 24 hours. The suspension was decanted and the snails were rinsed with running tap water and maintained for another 24 hours for possible recovery. All groups of the snails were observed within 24 hours for possible mortality at various concentration of the inoculums, mortality in each concentration were recorded and the mortality rate in percentage were calculated

#### **CERCARICIDAL ACTIVITY TEST**

*Biomphalaria pfeifferi* were individually placed in shading vials containing 5ml of aged water and were exposed to sunlight for 30 minutes, the emerging cercaria were pulled out and counted with the aid of microscope. Beakers containing 250ml of 5, 10, and 15mg/ml of the extract were prepared and set up in duplicates, the cercaria was observed with the aid of microscope. Similarly, 6 beakers contained 250ml of aged water was set up in duplicates. After exposing the cercaria for 2-4 hours to these concentrations. In each of the beakers one mice was placed through direct contact with the cercaria in the aged water containing the cercaria and allowed to stay for 40 minutes for exposure by paddling method (through skin penetration of the legs). After 40 minutes of exposure the mice were returned to their cages. Control mice was exposed to the same number of cercaria that were not exposed to the seed extract. Birrie, H. *et al.*, (2008).

After 45 days post-exposure, the infected mice were euthanized and dissected, the stool sample was collected from the large intestine.

#### **FORMOL-ETHER SEDIMENTATION TECHNIQUE**

##### **Procedure for Formal Ether Sedimentation Technique**

1. Hand gloves were worn while handling stool specimens. In a suitable container, a

portion of stool specimen was thoroughly mixed into 10mL of saline solution. The emulsion was filtered through muslin cloth into a conical centrifuge.

The suspension was centrifuge at relative centrifugal force (RCF) of 600g (about 2000 rpm) for no less than 10 minutes. The suspension yielded about 0.75mL of sediment. formalinized feces.

The supernatant was decanted and the sediment was washed thoroughly with 10ml saline solution. Centrifuged again and repeated washing until supernatant was clear.

After the last wash, the supernatant was decanted, 10ml of 10% formalin was added to the sediment. Mixed and let stood for 5 minutes to effect fixation. 1 to 2 ml of ethyl acetate was added, the tube was corked with a stopper and shook vigorously.

Centrifuged at 450g RCF (about 1500 rpm) for 10 minutes. Four layers was indicated.

1. a top layer of ethyl acetate;
2. plug of debris;

3. layer of formalin; and
4. sediment

The Freed debris plugged from the side of the tube by ringing with an applicator stick. Carefully decanted the top three layers.

With a pipette, the remaining sediment was mixed with the small amount or remaining fluid and transferred one drop each to a drop of saline and iodine on a glass slide. Covered with a coverslip and examined microscopically for the presence of parasitic forms.

## RESULT

TABLE 1: PHYTOCHEMICAL SCREENING OF *B. aegyptiaca* LEAVES, FRUITS AND ENDORCAP

Phytochemicals	Leaves	Fruits	Endocarp
Alkaloids	+	+	-
Saponins	+	+	+
Flavonoids	+	+	-
Tannins	+	+	+
Glycosides	-	-	-
Phenols	+	-	+
Steroids	+	+	-

Key: + =Positive - = Negative

TABLE 2: MOLLUSCICIDAL ACTIVITY TEST AGAINST *B. pfeifferi*

Inoculation Time (minutes)	Concentration of extract (ug/ml)	Number of tested snails	Number of death snails	Mortality rate (%)
60	10	10	1	10
90	15	10	3	30
120	20	10	3	30
150	25	10	3	30
180	30	10	5	50
210	35	10	6	60
240	40	10	7	70
470	45	10	7	70
300	50	10	9	90
330	55	10	9	90

Table 3: Cercaricidal activity of *B. aegyptiaca* leaves, fruits, and endocarp against infected mice

Sample	Number of infected Mice	Conc. of extract (ug/ml)					LC <sub>50</sub>
		10	15	20	25	30	
Leaves	20	2	4	5	4	5	0.0726
Fruits	20	0	3	5	6	6	0.0531
Endocarp	20	0	0	0	2	5	0.0426

## DISCUSSION

The present study showed that aqueous extracts of *Balanites aegyptiaca* leaves extract possess Molluscicidal properties. Their activities are time and concentration-dependent. Between the test snail hosts of trematodes, *Biomphalaria pfeifferi* was found susceptible to the plant extract than aqueous extracts of fruits

followed by endocarp extract which showed the lowest molluscicidal within and after 24 hours of exposure period. Previous investigators also reported similar observations Vijay, (2010); El-Sheikh, (1994); (Adewunmi and Sofowora, 1980).

In conformity with the work of Brimer *et al.*, (2007), the varying potencies of each plant part may be due to the

differences in the concentration and/or the type of the active ingredient(s) present in each part. In this observation, aqueous leaves extract were more potent than the fruit and endocarp extract, this may be due to the high concentrations of saponins in the leaves, and also presence of high amount of steroids in the fruit Bah *et al.*, 2006; Brimer *et al.*, (2007). Therefore, the presence of these two ingredients in the aforementioned plant parts might be the most probable reason for the high molluscicidal property of leaves over the stem bark and roots.

The cercaricidal potency of *B. aegyptiaca* extract of leaves, fruit and endocarp showed potency in the cercaricidal test conducted shows that sixty mice were exposed to cercaria and all became infected. The presence of schistosomes eggs were observed through microscopic examination. Upon oral administration of the extract, it could be deduced that at 20mg/ml and 30mg/ml of the leaves extract became effective to the eggs of the schistosomes found in the blood of the mice, 15mg/ml and 25mg/ml were effective against four (4) mice while 10mg/ml was effective against 2 mice. This in line with work of Abozeid *et al.*, which shows that aqueous and methanol extract of *punica granatum* were lethal 100% of cercaria at 25mg and 30mg concentration respectively.

## CONCLUSION

From this research work, it can be concluded that leaves extract of *Balanites aegyptiaca* were more effective to molluscicides and cercaricides against *Biomphalaria pfeifferi* and infected mice, which if modified and synthesized could be used as the effective treatment of schistosomiasis particularly to rural dwellers.

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