

Full Length Research Paper

## ***Phytolacca octandra* (L.), *Phytolacca dodecandra* (L'Herit) and *Balanites aegyptiaca* (L.) extracts as potential molluscicides of schistosomiasis transmitting snails**

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Received 23 August, 2016; Accepted 24 October, 2016

Schistosomiasis is a widespread parasitic infection whose intermediate host is aquatic snails and affects more than 250 million people worldwide. Although control of the snails with synthetic molluscicides is possible, it is not greatly preferred due to concerns of environmental toxicity and the relatively high cost of the chemicals. Conversely, organic plant-derived molluscicides are a better alternative that can be used to reduce the incidence of the disease. The objective of this study was to evaluate the molluscicidal activity of the plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*. The major parts of the whole plant (berries, leaves, stems and roots) were collected, air dried to constant weight, macerated to a fine powder and extracted separately using methanol in soxhlet apparatus. The extracts were screened for activity using brine shrimp lethality test and thereafter tested for molluscicidal activity. There was no significant difference observed in the activity of the plant parts studied and of the three plant species in the brine shrimp lethality test. Similarly, no significant difference in molluscicidal activity of the plant parts studied and in the three plants against *bulinus* snails was detected. It was concluded that that the three plants can be used in the control of schistosomiasis transmitting snails.

**Key words:** Schistosomiasis, molluscicidal, *Bulinus* snails, brine shrimp, *Phytolacca octandra*, *Phytolacca dodecandra*, *Balanites aegyptiaca*

### INTRODUCTION

Globally, schistosomiasis is the most devastating tropical disease after malaria and intestinal helminthiasis (Jenkins-Holick and Kaul, 2013) with Steinmann et

al. (2006) stating that in terms of number of people at risk and those infected, it is second to malaria. The disease is parasitic and caused by any of five species of

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*Schistosoma* trematodes, giving rise to acute and chronic diseases with clinical manifestations due to urinary (*S. haematobium*), intestinal (*S. mansoni*), or hepatosplenic involvements (Oliveira-Filho and Paumgarten, 2000). Steinmann et al. (2006) reported that more than 700 million people are at risk of infection whereas 207 million people are infected with the disease. According to World Health Organisation (WHO) (2016), the disease has been reported in 78 countries, whereby preventative chemotherapy is required in 52 endemic countries with moderate to high transmission. It is estimated that 90% of those requiring treatment live in Africa (WHO, 2016) and it is responsible for at least 200,000 deaths annually in sub-Saharan Africa (Jenkins-Holick and Kaul, 2013).

Schistosomiasis is regarded as a disease of poverty, occurring in areas that are remote and poverty-stricken with little or no safe water or sanitation and limited health care (Bruun and Aagaard-Hansen, 2008). Bruun and Aagaard-Hansen further stated that it is a disease classified by WHO as neglected by local, national and global actors. The lifecycle of the flatworms that cause human schistosomiasis involves a sexual stage in the human and an asexual stage in the freshwater snail host (Jenkins-Holick and Kaul, 2013). There are four main strategies to destroy the parasites: killing the worms in humans by anthelmintic drugs, killing the intermediate host snails by chemical (molluscicides) or biological control agents, stopping people infecting snails by preventing contamination of freshwater bodies with human waste and finally stopping infection of man by keeping people out of infested water bodies (Knopp et al., 2012).

Nicosamide is so far the only WHO recommended molluscicide but has disadvantages of requiring multiple applications for total elimination of the snails, making it time consuming and less cost effective (Inobaya et al., 2014). Oliveira-Filho and Paumgarten (2000) observed that although nicosamide is effective and less hazardous to environment and human health when compared with other molluscicides, it is costly for developing countries and is toxic to some non-target species like bacteria, algae, and first instar larvae of mosquitoes. Various researches have evaluated the effectiveness of plant-derived molluscicides for instance, Yadav and Singh (2011) noted that when the latex powder of *Euphorbia hirta* is used together with other substances (rutin, betulin, taraxerol and ellagic acid) it has potent molluscicidal activity on *Lymnaea acuminata* and *Indoplanorbis exustus* snails possibly due to neurotoxicity. *Euphorbia milii* latex was found to be a rather potent and selective plant molluscicide that is nontoxic to bacteria, algae, and first instar larvae of mosquitoes (Oliveira-Filho and Paumgarten, 2000).

Yadav and Singh (2014) found that sub-lethal exposure of *Jatropha gossypifolia* latex, leaf and stem bark and their different combinations with betulin, ellagic acid, rutin and taraxerol caused a significant reduction in survival

and hatchability of snail *Lymnaea acuminata*, killing the snails and rendering them sterile. The objective of this study was to evaluate the molluscicidal activity of the plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*.

## MATERIALS AND METHODS

### Description of plants

*Balanites aegyptiaca* (Fam. Balanitaceae) is an indigenous tree or shrub up to 12 m high with yellowish-grey to dark brown vertical cracked bark. Shoots have strong green spines up to 9 cm long, usually alternate and opposite to petioles of the leaves. Leaves are bifoliate with obovate folioles, pubescent underneath in young and glabrous in mature leaves. Flowers are green-yellowish, a little over 1 cm in diameter, solitary or fasciculate with cymes born at the base of leaves or spines. The fruit is an ellipsoid drupe, 3 to 4 cm long, turning from green to yellow when mature with a brown, ellipsoid hard stone surrounded by yellowish edible pulp (Food and Agriculture Organisation, 2016). *Phytolacca dodecandra* (Fam. Phytolaccaceae) is an indigenous climbing or scrambling dioecious, semi-succulent shrub or liana with glabrous stem. Leaves are petiolate, alternate, simple, entire and exstipulate with ovate blade, rounded base and acute glabrous apex. Inflorescence is axillary or terminal raceme. Flowers are unisexual, 5-merous; male flowers with narrowly oblong sepals, apetalous, 10 to 20 stamens in 2 whorls and a rudimentary ovary; female flowers have oblong to ovate sepals, apetalous, rudimentary stamens, with superior ovary of 4 to 5 carpels, curved styles 1 to 2 mm long and linear stigmas. Fruits are in clusters of 4 to 5, 1-seeded berries with kidney-shaped shiny black seeds. (Prota, 2016).

*Phytolacca octandra* (Fam. Phytolaccaceae) is a bushy perennial, up to 1 m high believed to have been introduced from South America but now naturalised in Kenya. Leaves are alternate, narrowly elliptic-lanceolate, hairless, margin entire and petiole to 2 cm long. Inflorescences are in terminal or lateral, leaf-opposed racemes, 4 to 14 cm long. Flowers are bisexual, white to yellowish-green with 8 stamens in one whorl. Fruits are depressed globose, mostly 6-8-lobed, 5-8 mm in diameter, hairless, dark purple to black when ripe (Flora of Zimbabwe, 2016).

### Collection of plants and snails

The plants *P. dodecandra* and *P. octandra* were collected in Njoro area of Nakuru County whereas the plant *Balanites aegyptiaca* samples were collected in Kitui County. The plants were identified at Egerton University. Snails were collected in Timboni dam in Kilifi County. The dam was chosen as it had a sandy beach, established vegetation such as water lilies and other floating vegetation which offer an ideal environment for the *Bulinus* spp. snails. The infection caused by *S. haematobium* or urinary schistosomiasis is very common in this area especially among school going children who swim and play in the infected waters of the dam. After collection, the snails were transported in a beaker, containing wet cotton wool to prevent dehydration, to the institute of Primate Research Malacology Laboratory at Fort Jesus, Mombasa, where an indoor culture was established. Each aquarium measuring 45 by 32 cm and 16 cm deep, made of polycarbonate material was used whose inside surfaces were rough to provide a gripping surface for the snails. To each aquarium was put approximately 5 litres of dechlorinated water. The bottom was lined with about 2 cm deep sand substrate. Aeration was by a whisper number 100 air pump, which was operated at low pressure to create minimal turbulence in the aquaria. The room temperature was maintained between 26

and 27°C. The collected snails were fed with lettuce and kept in these aquaria for 24 h for acclimatisation process before they could be used for the bioassay procedure.

### Preparation of plant materials

The plant materials were separated into berries, leaves, stem and roots and each part was packed in a clear labelled plastic bag. The parts were air dried for a period of six weeks after which the dried materials were ground using a hammer mill and the powdered samples were packed in clear labelled plastic bags ready for extraction.

### Extraction of the plant material

A total of 250g of each grounded sample was extracted using methanol in a soxhlet apparatus for a period of eight hours. The solvent was removed in vacuo to give the concentrated methanol extract, which was used for bioactivity test, and the rest dried in an evacuated dessicator.

### Bioassay

The World Health Organisation guidelines were followed in the bioassay tests. Each extract was tested at 200, 100 and 50 mg per litre of dechlorinated water. Three plastic containers plus one control were prepared for each extract concentration. Five acclimatised snails were placed into each of these containers with a total volume of 200 ml of the test extract solution. The snails were exposed to the test solution for 24 h at room temperature and normal diurnal lighting. The extract was decanted after 24 h and the snails rinsed twice with a stream of dechlorinated water. The snails were then fed with lettuce and left in clean water for a further 24 h in order to recover. Snails were considered dead if they remained motionless, not feeding, no heartbeat when observed under a microscope or shell looked discoloured at the end of the period. Control snails were not exposed to the test extract solution.

### Isolation and purification of active compounds

The crude extracts of the different parts of the different plants were isolated and purified as follows. They were dissolved to give an aqueous solution. The solvents used were distilled water, *n*-butanol, methanol and diethyl ether which were used in the case of *P. octandra* and *P. dodecandra* berries and cold methanol was used with all other different plants' parts. Filtering gave a brown precipitate for *P. octandra* stem and white crystals for the other plant parts after washing with cold methanol, but also with pet-ether and ethyl acetate for *P. octandra* stem. The melting point was determined using a Sanyo melting point apparatus. Thin layer chromatography (TLC) analysis of the crystals was done with methanol and silica gel. Antimony chloride in concentrated hydrochloric acid (HCL) was used as the spraying reagent. Various chemical tests done included: test for triterpenoids using Liebermann-Buchard reagent (acetic anhydride in concentrated sulphuric acid), alkaloids using Dragendorff reagent (Bismuth nitrate and Potassium iodide in water) and test for saponins. Elemental analysis was carried out using the CE 440 Elemental Analyser Exeter Analytical instrument.

### Screening the plants' extracts by use of brine shrimp lethality test

The extracts were tested at 1000, 100 and 10 ppm. Five test tubes

and one control were prepared at each concentration for a total of 18 test tubes per extract. Fifty milligrams of the dried extract were weighed and dissolved in 5 ml of methanol. From this solution 500, 50, and 5 µl were transferred into test tubes corresponding to 1000, 100 and 10 ppm, respectively using a micro-pipette syringe. The solvent was evaporated using a blow drier. Extracts that were insoluble in methanol were dissolved in dimethylsulfoxide (DMSO)-AR and up to 50 µl per 5 ml of brine were used to avoid DMSO toxicity affecting the results. The prepared extracts were stored in a dry place.

A saline solution was prepared by dissolving 33 g of sea salt in one litre of water. Ten millilitres of this solution was transferred into a petri-dish on which two milligrams of brine shrimp eggs were sprinkled. The brine shrimps eggs were placed in an incubator and allowed to hatch for 24 h. After hatching ten brine shrimps larvae were transferred into each prepared extract tube using a Pasteur pipette. Five millilitres of the saline solution were then added into each of the test tubes containing the larvae and the extracts. The number of surviving larvae were then counted and recorded. From the data the % mortality was calculated using Abbott's formula after correction for control. The formula is given below:

$$\% \text{ Mortality} = \frac{(\text{sample mortality} - \text{control}\% \text{ mortality})}{100 - \text{control}\% \text{ mortality}} \times 100$$

### Statistical analyses

Statistical analysis was done to determine LD<sub>50</sub> using Probit procedure. These values were subjected to analysis of variance (ANOVA) using general linear model (GLM) procedure and the means separated by using Duncan's Multiple Range Test (DMRT). SAS proprietary software release 8.1 (1999 to 2000) was used in these analyses. Correlation was done on the mean LD<sub>50</sub> and the percent mortality for the three plants and their parts.

## RESULTS

### Percent yield of the plant materials using Soxhlet apparatus

Soxhlet extraction method gave the highest per cent yield for the berries of *P. octandra* and *P. dodecandra*, and the lowest for *B. aegyptiaca* berries (Table 1). However, analysis of variance showed no significant ( $P > 0.05$ ) difference in mean percent yields of the three plants.

### Brine shrimp assay

#### *Mean percent mortality for the berries, leaves, stem and roots of the plants*

Calculation for the mean percent mortality after correction for control for the different plants parts showed that the leaves of *P. octandra* had the highest percent mortality when compared to other plants (*P. dodecandra* and *B. aegyptiaca*) parts, whereas the lowest mean percent mortality was recorded in the stem of *B. aegyptiaca* (Table 2). The berries with the highest percent mortality were those of *P. octandra*. The stem of *P. octandra* was

**Table 1.** Percent Yield of the plant materials of the three plants.

Plant	Plant part	% yield	Mean % yield
<i>P. octandra</i>	Berries	30.95	15.27 <sup>a</sup>
	Leaves	18.56	
	Stem	8.56	
	Roots	8.24	
<i>P. dodecandra</i>	Berries	30.89	14.04 <sup>a</sup>
	Leaves	7.92	
	Stem	11.53	
	Roots	5.82	
<i>B. aegyptiaca</i>	Berries	2.75	9.76 <sup>a</sup>
	Leaves	14.76	
	Stem	11.08	
	Roots	10.45	

<sup>a</sup>Means with the same superscript are not significantly different at 5 % level

**Table 2.** Mean percent mortality for the berries, leaves, stem and roots of each of the three plant species.

Plant part	Mean % mortality $\pm$ SEM*		
	<i>P. octandra</i>	<i>P. dodecandra</i>	<i>B. aegyptiaca</i>
Berries	27.00 $\pm$ 5.42	22.00 $\pm$ 9.37	24.89 $\pm$ 7.35
Leaves	37.67 $\pm$ 2.83	18.00 $\pm$ 2.34	35.11 $\pm$ 8.57
Stem	32.5 $\pm$ 6.21	21.44 $\pm$ 6.59	11.23 $\pm$ 2.89
Roots	32.19 $\pm$ 4.49	32.89 $\pm$ 3.02	36.02 $\pm$ 2.20

\*standard error of the mean

**Table 3.** Mean LD<sub>50</sub> for the berries, leaves, stem and roots for the three plants species.

Plant part	Mean LD <sub>50</sub> $\pm$ SEM		
	<i>P. octandra</i>	<i>P. dodecandra</i>	<i>B. aegyptiaca</i>
Berries	2.2627 $\pm$ 0.7097	2.9865 $\pm$ 0.0243	2.6839 $\pm$ 0.1837
Leaves	2.4181 $\pm$ 0.1923	2.5397 $\pm$ 0.1466	2.6770 $\pm$ 0.4604
Stem	2.4197 $\pm$ 0.3446	2.8193 $\pm$ 0.2517	2.8523 $\pm$ 0.0954
Roots	2.5672 $\pm$ 0.1815	2.5332 $\pm$ 0.1089	2.0931 $\pm$ 0.00

found to have the highest mean percent mortality whereas the roots of *B. aegyptiaca* were found to have the highest mean percent mortality. LD<sub>50</sub> for the berries, leaves, stem and roots for the three plants species LD<sub>50</sub> is the dose that kills 50% of the organisms exposed to the test substance within 24 h of exposure. Potent substances therefore, give lower LD<sub>50</sub> values. Analysis of variance showed no significant difference ( $P > 0.05$ ) in the mean LD<sub>50</sub> values for the different plant parts that is the berries,

leaves, stem and roots of the three plants (Table 3). The best mean LD<sub>50</sub> value for the berries, leaves and stem were recorded for the plant *P. octandra* extracts. However, these were not significantly different ( $P > 0.05$ ) from the LD<sub>50</sub> values recorded for berries, leaves, stem of *P. dodecandra* and *B. aegyptiaca*. The plant *B. aegyptiaca* recorded the best mean LD<sub>50</sub> value for the roots extract although it is not significantly different ( $P > 0.05$ ) from the LD<sub>50</sub> value of roots recorded for *P.*

**Table 4.** LD<sub>50</sub> for the berries, leaves, stems and roots of the plants *P. octandra*, *P. dodecandra* and *Balanites. Aegyptiaca*.

Plant part	Mean LD <sub>50</sub> ± SEM		
	<i>P. octandra</i>	<i>P. dodecandra</i>	<i>B. aegyptiaca</i>
Berries	1.4520±0.1011	1.1427±0.1356	0.9609±0.0020
Leaves	0.8846±0.0742	0.8749±0.0356	0.7975±0.0719
Stem	1.0438±0.0093	0.7305±0.1349	0.9415±0.0062
Roots	1.0310±0.1205	0.8885±0.0742	1.2482±0.3551

*octandra* and *P. dodecandra*.

### Comparison of mean LD<sub>50</sub> and mean percent mortality

Correlation analysis performed on the mean LD<sub>50</sub> and the percent mortality for the three plants species and their parts showed significant negative correlation ( $P = 0.0345$ ,  $r = -0.61178$ ). This means that an increase in the LD<sub>50</sub> value may be associated to a greater extent with a decrease in the percent mortality for the plants and plant parts studied.

### Molluscicide test: LD<sub>50</sub> values for the plant parts of the three plant species

Analysis of variance showed no significant difference ( $P > 0.05$ ) in the mean LD<sub>50</sub> values for the different plant parts of the three plants (Table 4). The best mean LD<sub>50</sub> value for the berries and leaves were recorded in *B. aegyptiaca*, whereas *P. dodecandra* recorded the best mean LD<sub>50</sub> for the stem and roots. However, these were not significantly different ( $P > 0.05$ ) from the mean LD<sub>50</sub> values recorded for the berries, leaves, stem and roots of *P. octandra*, the berries and leaves of *P. dodecandra* and the stem and roots of *B. aegyptiaca*.

## DISCUSSION

From the experimental data, the regenerative parts of the plants appear to be equally active as any other part of the plants since no significant difference ( $P > 0.05$ ) exists among the parts. This makes the regenerative parts suitable for development of new molluscicides since their exploitation would not endanger the plants which grow in many parts of the country. In the experiment, the extracts were tested within the range of between 1000 to 10 mg/L instead of the recommended 100 to 10 mg/L (WHO, 1961). This is due to the low activity recorded in the recommended range which could probably be as a result of very low concentration of saponins in the extracts as reported by Treyvaud et al. (2000). The mode of action of the extracts is probably haemolysis since the

colourless extract solution turned reddish in colour six hours after exposure of the snails to the extract. These findings are consistent with those of Harborne (1984). In Andwa, Ethiopia a five year study of the effect of the application of *P. dodecandra* berries in local streams lead to the reduction of snail population which resulted to reduced prevalence of *S. mansoni* in children from 50% at the start of the project to 7% at the end and a reduction of incidence in the entire population of the area from 63 to 34% (Lemma, 1983). Similarly, Molla (2011) found that different plant parts of *Balanites aegyptiaca* were effective in reducing the population of schistosomiasis-transmitting snails. The use of these plants is supported by Lemma (1983) who reported that application of *P. dodecandra* berries in the control of the schistosomiasis-transmitting snails had no obvious adverse effects on other microflora and fauna of treated streams. From the results of molluscicidal activity, no significant ( $P > 0.05$ ) difference exist between the plant parts (the berries, leaves, stem and roots) and the three plants studied. These results are consistent with those obtained in the Brine shrimp lethality in that no significant difference in activity was recorded between the plant parts and between the plants studied. These results are supported by earlier findings of the World Health Organization that the effect of a potential molluscicide on fish and shrimp can be used to determine the effectiveness of a compound (WHO, 1961).

## CONCLUSIONS AND RECOMMENDATIONS

Since the regenerative parts are equally active, communities would be encouraged to use these parts which would lead to conservation of the plants in the country. Similarly, as there is no significant difference in the molluscicidal activity of the three plants studied, communities should be advised to conserve and utilize any of these plants that can grow well their locality for controlling schistosomiasis-transmitting snails.

It is possible to monitor active extracts for molluscicidal activity using Brine shrimp lethality test because it has the advantages of being rapid (24 h following introduction of the shrimps), inexpensive, and simple (for example no aseptic technic required). It also utilizes a large number of organisms for statistical consideration and requires no

special equipment and a relatively small amount of sample (20 mg for screening initial extracts at 1000 ppm, lesser amounts for active fractions). From this study, the following are recommended as areas of further research:

1. Testing the extracts and compounds against genus *Biomphalaria* snails which are intermediate host of fecal schistosomiasis.
2. Evaluating the miracidiacidal and cercariacidal properties of the extracts and compounds.
3. Spectroscopic (x-ray crystallography, NMR, Mass) analysis on the isolated compounds to determine their structure.

### Conflict of interest

The authors have not declared any conflict of interests.

### ACKNOWLEDGMENTS

The authors are grateful to Egerton University and the Institute of Primate Research Malacogy Laboratory at Fort Jesus, Mombasa in which the study was carried out.

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