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Molluscicidal Activity of Selected Plant Extracts in Kenya

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Abstract
Schistosomiasis is a snail-borne infection and one approach to the control of schistosomiasis is elimination of the intermediate host. The use of synthetic molluscicides is becoming unpopular and as a result, plant molluscicides have received considerable attention in search for cheaper alternatives to synthetic molluscicides. In this study, the molluscicidal activity of aqueous and ethanol extracts of selected plants against adult and juveniles of *Biomphalaria pfeifferi* snails was investigated. Dried plant materials from *Ocimum americanum, Sonchus luxurians, Aloe secundiflora, Bridelia micrantha* and *Croton megalocarpus* were ground into powder and extraction done using ethanol and water. Phytochemicals were tested which include; flavonoids, saponins, tannins, alkaloids, glycosides, steroids and triterpenes. Ten adult and ten juvenile *Biomphalaria pfeifferi* snails were exposed to serial dilutions of 5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l of the aqueous and ethanol plant extracts. The exposure period was 48 h. The LD_{50} value of the plant extracts was determined. The lethal dose, LD_{50} of *B. micrantha* against the adult snails was 24.98 mg/l for the aqueous extract and 19.01 mg/l for the ethanol extract. The lethal dose, LD_{50} of *B. micrantha* against the juvenile snails was 22.860 mg/l for the aqueous extract and 26.30 mg/l for the ethanol extract. The other extracts from the other plants were found to have a LD_{50} value of above 100 mg/l.Generally, only *B. micrantha* which had molluscicidal activity against adult and juvenile snails. *B. micrantha* extracts, that were found to have molluscicidal activity, were screened for their miracicidal and cercaricidal activity against *Schistosoma mansoni* miracidia and cercariae using concentrations of 5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l. The exposure period was 1h. *Bridelia micrantha* aqueous extract had a LD_{50} value of 11.108 mg/l on the *S. mansoni* miracidia and a LD_{50} value of 4.465 mg/l on *S. mansoni* cercariae. The ethanol extract was found to have a LD_{50} value of above 40 mg/l for the miracidia and the cercariae. From this study, *B. micrantha* demonstrated molluscicidal activity against *Biomphalaria pfeifferi* snails and miracicidal and cercaricidal activity against *Schistosoma mansoni* miracidia and cercariae.
Keywords: Molluscicidal, Phytochemicals, Miracicidal, Cercaricidal.

1. Introduction

Schistosomiasis is one of the most important public health problems after malaria (Mohammed, 2009). It is one of the most widespread parasitic infections and the third most prevalent parasitic disease in the world in terms of overall morbidity, socioeconomic and public health importance. It is the most prevalent of water borne and parasitic disease infecting over 240 million people (WHO, 2013). In Kenya, it is estimated that over one million people are infected with the disease (WHO, 2013). The social-economic and health effects of schistosomiasis cannot be underestimated. Infected children have retarded growth and poor school performance. The work capacity of rural inhabitants is severely reduced due to weakness and lethargy caused by the disease (Kloos & McCullough, 1987).

One approach to the control of the disease is the elimination of the intermediate host responsible for its transmission. The use of synthetic molluscicides is becoming unpopular owing to their high cost and environmental pollution. Plant molluscicides have received considerable attention in the search for cheaper alternatives to chemotherapy, and synthetic molluscicides in schistosomiasis control. Currently, only one molluscicide, namely, niclosamide, is available for snail control and only one drug, praziquantel is available for all forms of the disease (Fenwick *et al.,* 2006).

Niclosamide is costly, toxic to non - target organisms, has a low dispersibility and precipitates rapidly (Jose' 2003) while resistance to praziquantel has been demonstrated in some schistosomiasis endemic areas of the world (Fenwick *et al.,* 2006). Plant molluscicides can provide a source of low cost, locally produced, safe and effective molluscicide (Sigh *et al.,* 1996). They are environmentally acceptable (El-Sawy *et al*., 1981) and may provide an opportunity of incorporating snail control into the community - based Primary Health Care (PHC) approach of schistosomiasis control (Fenwick *et al*., 2006). Among the plants of great interest are those which contain large quantities of saponins. Saponins possess high toxicity against cold blood organisms including snails (Hostettmann *et al.,* 2000).

The present study aimed to evaluate molluscicidal, miracicidal and cercaricidal effect of extracts of plants with molluscicidal and antihelminthic properties as monitored by determination of survival of snails *(Biomphalaria pfeifferi*) and *Schistosoma mansoni* miracidia and cercariae.

2. Materials and Methods

2.1 Plant Collection

The plants used were selected on the basis of information by traditional healers to be useful in the treatment of helminthes in endemic areas. The plants used were *Bridelia micrantha*, Croton *megalocarpus*, *Sonchus luxurians*, *Ocimum americanum* and *Aloe secundiflora*. The three whole plants; *Ocimum americanum, Sonchus luxurians,* and *Aloe secundiflora* and the barks of *Bridelia micrantha* and *Croton megalocarpus* were collected from their natural habitat and dried under shade for two months.

2.2 Preparation of Plant Extracts

The dried plants were ground into powder and stored at room temperature. Crude plant extracts were prepared using ethanol and distilled water. To obtain the ethanol extracts, 100 g of the powder from each plant were soaked in 500 ml of ethanol for one day (24 h). The solutions were filtered using filter paper (Whatman No. 1) and subjected to freeze drying using laboratory freeze drier. The freeze dried materials constituted the ethanol extracts (Das *et al*., 2010). To extract the polar materials, 100 g of the powder from each plant were soaked in 500 ml of distilled water for three days (72 h). The solutions were filtered using filter paper (Whatman No. 1) and allowed to freeze. The frozen material was then subjected to freeze drying using laboratory freeze drier. The freeze dried materials constituted the aqueous extracts (Das *et al*., 2010).

2.3 Phytochemical Screening

Phytochemical screening was performed using standard procedures (Trease & Evans, 1989; Siddiqui & Ali, 1997; Harborne, 1998) to test for triterpenes, sterols, flavonoids, glycosides, saponins, tannins and alkaloids.

2.4 Snail Collection and Maintenance

Biomphalaria pfeifferi adult snails collected from canals in Mwea irrigation scheme were screened for any infection. Those not infected were allowed to lay eggs. The eggs were allowed to hatch and develop for one week into juveniles. The adult snails and the juveniles were used for molluscicidal activity.

2.5 Molluscicidal Activity

The adult snails were placed in distilled water for 24 h being fed on dried lettuce. They were then placed in the extracts for 48 h without being fed; 5 mg/litre, 10 mg/litre, 20 mg/litre and 40 mg/litre of both the aqueous and the ethanol extracts were used. Niclosamide served as the positive control at a concentration of 0.1 mg/l (McCullough, 1992) and distilled water served as the negative control. After the 48 h, the snails were transferred into distilled water for a recovery period of 24 h. Duplicate set – ups were set for each of the different concentrations made. Identification of dead snails was made based on the snail's mobility. The same procedure was repeated using the juvenile snails.

2.6 Miracicidal and Cercaricidal Activity

Bridelia micrantha aqueous and ethanol extracts which were the only ones that were found effective against both adult and juvenile snails were used to test miracicidal activity. Miracidia were hatched from eggs from faecal samples of baboons with *Schistosoma mansoni* chronic infection.

Different concentrations: 5 mg/litre, 10 mg/litre, 20 mg/litre and 40 mg/litre of both the aqueous and the ethanol extracts of *Bridelia micrantha* were used for miracicidal tests. Adult snails were infected with miracidia and maintained for four weeks to allow development of miracidia to cercariae. After four weeks they were then placed in a beaker and placed under 200 watts lamp shielded with glass to shed cercariae (Yole *et al*., 1993). Concentrations of 5 mg/litre, 10 mg/litre, 20 mg/litre and 40 mg/litre of both the aqueous and the ethanol extracts of *Bridelia micrantha* were used for cercaricidal tests. Lethal dose (LD_{50}) value of the extracts on the snails, miracidia and cercariae was determined by means of probit analysis.

3. Results

3.1 Qualitative Analysis of the Crude Extracts

Alkaloids were detected in the ethanol and aqueous crude extracts of *Croton megalocarpus* and *Aloe secundiflora*, ethanol crude extracts of *Ocimum americanum* and *Bridelia micrantha* as well as aqueous crude extracts of *Sonchus luxurians.* Saponins were detected in the ethanol and aqueous crude extracts of *Ocimum americanum, Bridelia micrantha* and *Sonchus luxurians* as well as the aqueous crude extracts of *Croton megalocarpus* and *Aloe secundiflora*. Glycosides were detected in ethanol and aqueous crude extracts of *Ocimum americanum, Bridelia micrantha* and *Sonchus luxurians* as well as the aqueous crude extracts of *Croton megalocarpus* and *Aloe secundiflora*. All the phytochemicals tested were detected in *Ocimum americanum* and *Bridelia micrantha* ethanol extracts while alkaloids were not detected in their aqueous extracts. All the phytochemicals tested were also detected in aqueous extracts of *Croton megalocarpus* and *Aloe secundiflora*

while saponins and glycosides were not detected in their ethanol extracts. The tested phytochemicals were also detected in aqueous extracts of *Sonchus luxurians* while alkaloids were not detected in its ethanol extracts. In all the ethanol extracts tested, only in *Sonchus luxurians'* ethanol extract were alkaloids not detected.

3.2 Molluscicidal Activity

The various ethanol extracts had molluscicidal activity which was significantly different among the various treatments ($F_{6, 21}$=31.636; p<0.05). Only the comparison of *B. micrantha* ethanol extracts with niclosamide gave a sig. level, p>0.05 for both juvenile and adult snails at p=0.73 and p=0.51 respectively. Comparison of the other ethanol extracts with niclosamide gave a sig. level p<0.05. Hence, only the ethanol extracts of *B. micrantha* had a molluscicidal activity which was not significantly different from that of niclosamide.

Molluscicidal activity due to aqueous extracts was significantly different among all the treatments ($F_{6, 21}$=20.970; p<0.05). Only the comparison of *B. micrantha* aqueous gave a sig. level p>0.05 for both juvenile and adult snails at p=0.62 and p=0.53 respectively. Hence, only the aqueous extracts of *B. micrantha* had a molluscicidal activity which was not significantly different from that of niclosamide.

3.3 Miracicidal Activity

The various concentrations of the aqueous and ethanol extracts of *B. micrantha* had significantly different miracicidal activity within 60 minutes ($F_{4, 15}$ =4.579; p<0.05). P values obtained from the comparison of concentrations of the aqueous extracts with niclosamide were p>0.05. In the ethanol extracts comparison of 20 mg/l and 40 mg/l gave p. values p>0.05 i.e. p=0.921. Hence, all the concentrations of the aqueous extracts of *B. micrantha* (5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l) and the 20 mg/l and 40 mg/l of the ethanol extract of *B. micrantha* had a miracicidal activity similar to that of niclosamide.

3.4 Cercaricidal Activity

The various concentrations of the aqueous and ethanol extracts of *B. micrantha* had significantly different cercaricidal activity within 60 minutes ($F_{4, 15}$=3.949; p<0.05. The p values obtained after comparison of all the concentrations of the aqueous extract of *B. micrantha* with niclosamide were p>0.05. Only comparison of 40mg/l of ethanol extract of *B. micrantha* gave a p value p>0.05 i.e. p=0.407. Hence 5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l of aqueous extracts of *B. micrantha* together with 40 mg/l of the ethanol extract of *B. micrantha* had cercaricidal activity which was not significantly different from that of niclosamide.

3.5 LD_{50} Determination of the Extracts on Adult and Juvenile Snails

The aqueous extract of *B. micrantha* was found to have a LD_{50} value of 24.98 mg/l and the ethanol extract LD_{50} value 19.01 mg/l on the adult snails. The other extracts were found to have a LD_{50} value of above 100 mg/l.
The aqueous extracts of *B. micrantha* was found to have a LD_{50} value of 22.860 mg/l, while the ethanol extracts was found to have a LD_{50} value of 26.30 mg/l on the juvenile snails. The other extracts from the other plants were found to have a LD_{50} value of above 100 mg/l.

3.6 LD_{50} Determination for Miracicidal and Cercaricidal activity

Bridelia micrantha aqueous extract had a LD_{50} value of 11.108 mg/l on the *S. mansoni* miracidia and a LD_{50} value of 4.465 mg/l on the cercariae. The ethanol extract was found to have a LD_{50} value of above 40 mg/l for the *S. mansoni* miracidia and the cercariae.

4. DISCUSSION

4.1 Qualitative Analysis of the Crude Extracts

Tannins, flavonoids and steroids/triterpenes were detected in both ethanol and aqueous crude extracts of all the plants tested. Most methods exploit the property of most alkaloids to be soluble in organic solvents but not in water, and the opposite tendency of their salts (Manske, 1965). For this reason, the absence of alkaloids in the aqueous extracts of *Bridelia micrantha* and *Ocimum americanum* and yet they were present in their ethanol extracts may indicate that their occurrence in these plants is in form of salts of organic solvents. The absence of alkaloids in the ethanol crude extract of *Sonchus luxurians* and yet they were present in the aqueous extract may indicate that their occurrence in these plants is not in form of salts of organic solvents.

Saponins have special structural features and in general it is difficult to use a single technique for extraction of saponins. For this reason, the absence of saponins in the ethanol extracts of *C. megalocarpus* and *A. secundiflora* and yet they were present in their aqueous extracts may indicate that saponins from various sources have different extraction procedures (Yadav & Munin, 2011). Glycosides are generally water soluble but, some are soluble in alcohol. The absence of glycosides in the ethanol extracts of *Croton megalocarpus* and *Aloe secundiflora* may indicate that the glycosides in these plants are insoluble in alcohol.

4.2 Activity of the Plant Extracts on the Adult and Juvenile Snails

Bridelia micrantha extracts had the highest effect on the juvenile and adult snails. The significant value obtained after the analysis is p=0.000 which is p<0.05. Hence, the various ethanol and aqueous extracts had molluscicidal activity which was significantly different among the various treatments. On comparison to niclosamide, *B. micrantha* ethanol extract gave a significant level p>0.05 for both juvenile and adult snails; p=0.73 and p=0.51 respectively. Comparison of *B. micrantha* aqueous extracts with niclosamide gave a significant level p>0.05 for both juvenile and adult snails i.e. p=0.62 and p=0.53 respectively. Comparison of the other ethanol and aqueous extracts from the other plants with niclosamide gave a significant level p<0.05. The aqueous extract of *B. micrantha* was more effective (p value 0.53) than the ethanol extract (p value 0.51). The adult snails were more susceptible to *B. micrantha* extracts than the juvenile snails.

4.3 Miracicidal Activity of Bridelia micrantha

Analysis of significant value obtained is p=0.13 which is p< 0.05. Significant values obtained from the comparison of concentrations of the aqueous extracts with niclosamide were p> 0.05. (5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l gave p values of p=0.744, p=0 .949, p=0 .981 and p=1.000 respectively). In the ethanol extracts, comparison of 20 mg/l and 40 mg/l gave p values of p=0.921 (p>0.05). Hence all the concentrations of the aqueous extracts of *B. micrantha* (5mg/l, 10mg/l, 20mg/l and 40 mg/l), 20 mg/l and 40 mg/l of the ethanol extract of *B. micrantha* had a miracicidal activity similar to that of niclosamide.

4.4 Cercaricidal activity of Bridelia micrantha

Comparison of all the concentrations of the aqueous extract of *B. micrantha* with niclosamide was done. The p values were p=0.058, p=0.298, p=0.058 and p=0 .298 for 5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l respectively. Only comparison of 40mg/l of ethanol extract of *B. micrantha* gave a p value >0.05 (p=0.407). Hence the 5 mg/l, 10 mg/l, 20 mg/l and 40 mg/l concentrations of aqueous extracts and the 40 mg/l of the ethanol extract of *B. micrantha* had cercaricidal activity which was not significantly different from that of niclosamide.

4.5 LD_{50} Determination

B. micrantha aqueous extract was highly toxic on adult snails; LD_{50}<100mg/l while *S. luxurians, O. americanum, A. secundiflora* and *C. megalocarpus* aqueous extracts were moderately toxic; LD_{50}>100< 500mg/l. *B. micrantha* ethanol extract was highly toxic; LD_{50}<100mg/l, *O. americanum, A. secundiflora* and *C. megalocarpus* were moderately toxic; LD_{50}>100< 500mg/l while *S. luxurians* was non toxic to adult snails; LD_{50}>1000mg/l. The aqueous extract of *Bridelia micrantha* was found to have a LD_{50} value of 24.98 mg/l, while its ethanol extract was found to have a LD_{50} value 19.01 mg/l.

Exposure of the juvenile snails to the extracts revealed that, *B. micrantha* aqueous extract was highly toxic; LD_{50}<100mg/l, *O. americanum* was moderately toxic; LD_{50}>100< 500mg/l while *S. luxurians, A. secundiflora,* and *C. megalocarpus* were non toxic on juvenile snails; LD_{50}>1000mg/l. For the ethanol extracts, *B. micrantha* was highly toxic; LD_{50}<100mg/l, *A. secundiflora* was moderately toxic; LD_{50}>100< 500mg/l, *S. luxurians, O. americanum,* and *C. megalocarpus*, were non toxic on juvenile snails; LD_{50}>1000mg/l. The aqueous extract of *B. micrantha* was found to have a LD_{50} value of 22.86 mg/l, while the ethanol extracts of *B. micrantha* was found to have a LD_{50} value of 26.30 mg/l. For the juvenile snails the aqueous extract of *B. micrantha* was more efficacious with a LD_{50} value of 22.86 mg/l than the ethanol extracts, with a LD_{50} value of 26.30 mg/l.

Exposure of *Schistosoma mansoni* miracidia to *Bridelia micrantha* extracts revealed that the aqueous extract had a LD_{50} value of 11.108 mg/l, while the ethanol extract had a LD_{50} value of above 40 mg/l. The miracidia were more susceptible to the aqueous extract than to the ethanol extract. Exposure of *Schistosoma mansoni* cercariae to *Bridelia micrantha* extracts revealed that *B. micrantha* aqueous extract had a LD_{50} value of 4.465 mg/l, while the ethanol extract was found to have a LD_{50} value of above 40 mg/l. For the cercariae, the aqueous extract of *B. micrantha* was more efficacious with a LD_{50} value of 4.465 mg/l, than the ethanol extract, with a LD_{50} value of above 40 mg/l. Water is a universal extractor. The higher susceptibility of the miracidia and the cercariae to the aqueous extract than to the ethanol extract may be due to presence of some phytochemicals especially those extracted by water which have different structures form those extracted by ethanol (Sen *et al*., 1998b).

5. Conclusion

This study has been able to demonstrate significant molluscicidal, miracicidal and cercaricidal activity in *B. micrantha* which could be used in control of schistosomiasis. *B. micrantha* has prospects to warrant further research towards development of a molluscicide which may give solution to the control of schistosomiasis transmission by snails.

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