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Full Length Research Paper

Effect of ethanolic extract of *Dalbergia sissoo* plant parts on *Biomphalaria alexandrina* snail, the intermediate host of *Schistosoma mansoni*

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This study evaluated, using replicated laboratory bioassays, the toxicities of the ethanolic extracts of *Dalbergia sissoo* (family Leguminosae) fruits, leaves, roots and stem bark against adult *Biomphalaria alexandrina*, the snail intermediate host of *Schistosoma mansoni* in Egypt and their egg masses. Adult snails (6 to 8 mm in diameter) and viable 24 h-old embryonated egg masses were separately exposed to seven different concentrations (6.25 to 400 ppm) of extracts for 24 h. The LC_{50} and LC_{90} values of test extracts for adult snails and egg masses were calculated by probit analysis. The activities of the tested extracts were concentration-dependent. However, only the ethanolic extract of the fruits demonstrated significant activity on both adult snails and egg masses (24 h-LC_{90} value 34.4 and 38.6 ppm, respectively). Mortalities of eggs were manifested at the gastrula/exogastrula and or the pre-hatch snail stage of development. Ethanolic extract of the fruits was the most active with 100% mortality at 50 mg/l, followed by those of the leaves (at 100 mg/l), roots (at 200 mg/l) and stem bark (at 400 mg/l). Their respective 24 h-LC_{50} and LC_{90} values for *B. alexandrina* egg masses were 10.8 and 38.6 ppm, 18.5 and 68.3 ppm, 20.4 and 88.4 ppm, 36.8 and 144.6 ppm. The percentage of dead embryos at all stages increased with increasing concentration of extract. Lethality of the ethanolic extract of *D. sissoo* fruits to embryonated egg masses of *B. alexandrina* is an added advantage to its potential development for use as a plant molluscicide, as the overall efficacy of a molluscicide is greatly enhanced if it also shows significant toxicity towards snail eggs.

Key words: *Dalbergia sissoo*, *Biomphalaria alexandrina*, *Schistosoma mansoni*, egg masses.

INTRODUCTION

Human schistosomiasis is a parasitic disease caused by digenetic trematode species of the genus *Schistosoma* which co-habitate the venous plexuses of the mammalian viscera (Lockyer et al. (2003) and transmitted by fresh-water gastropod molluscs which serve as intermediate hosts. In the tropics and subtropics, schistosomiasis is the second most important parasitic disease after malaria in terms of prevalence, public health and socio-economic importance (WHO, 1993; Pointier and Giboda, 1999; Chitsulo, et al., 2000).

The World Health Organization recommends an integrated control of schistosomiasis (WHO, 1993). Thus, an appropriately targeted snail control using molluscicide (s) is an important preventive strategy which should be combined with carefully managed and directed chemo-therapy, ecological and biological control methods, as well as socio-economic improvements and advances in health education with community participation (Clark et al., 1997; Schall et al., 1998). Snail control in schistosomiasis is based on the rational assumption that elimination or reduction of snail population density below a certain critical threshold, would reduce transmission to a level at which the rate of new human infections, as measured by disease incidence, is significantly reduced or stopped altogether (WHO, 1993). In addition to controlling the adult snails, control of their egg masses would also result in their eventual decimation. It is thus advantageous, if a

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molluscicide also kills snail eggs (Kloos and McCullough, 1987).

The high costs of synthetic molluscicides such as niclosamide, their toxicities to non-target aquatic biota and even man Andrews et al. (1983), Abdel-Hamid (2003), Pieri et al. (1995), Rawi et al. (1996), as well as the complex organization required in their application, are a major setback to their continued use, especially in schistosomiasis control program. There is thus, the need for cheaper, environmentally friendly, biodegradable and readily available natural molluscicides from plants. Biologically active natural products abound in African medicinal plants (Marston et al., 1993).

Dalbergia sissoo (Family: Leguminosae), a deciduous tree, different parts of which are used in alternative medicine as anthelmintic Burkill (1985), and could offer greater promise for development as a plant molluscicide (Burkill, 1995).

This study aims to evaluate the toxicities of ethanolic extract of *D. sissoo* fruits, leaves, roots and stem bark on further development of the embryonated eggs of *B. alexandrina*.

MATERIALS AND METHODS

Plant materials

Fruits, leaves, roots and stem bark of *D. sissoo* were collected in the fresh state from the field and promptly transported to the laboratory for identification and authentication.

Preparation of extracts

All plant parts were air dried to relatively stable weights, chopped into bits and thereafter ground to moderately-fine powders (mesh size, 200µ), using an electric blender (Molyneux). Ethanolic extracts were prepared by steeping 40 g of powdered plant material in 90% ethanol for 48 h, followed by filtration. Extracts were concentrated to dryness by evaporation, under reduced pressure, in a rotary evaporator (NYC R-205D) at temperature below 45°C. Stock solutions of the extracts were prepared fresh, by dissolving 4 g of dry extract in 20 ml dimethylsulfoxide (DMSO) and then completing to 100 ml volume with dechlorinated tap water. Serial dilutions of the stock solutions with the appropriate volumes of dechlorinated tap water afforded final assay solutions of 400, 200, 100, 50, 25, 12.5, 6.25 mg/l as required. Final concentration of DMSO in both test and control solutions was 0.5%.

Assay for adult *B. alexandrina* snails

The efficiency of ethanolic extracts of various parts of the plant against adult *B. alexandrina* snails was determined according to the standard procedure recommended by WHO (1993). A series of concentrations that would permit the computation of LC_{50} and LC_{90} values were prepared by probit analysis according to Finney (1971).

Assay for ovicidal activity

The 24 h old egg masses laid by first generation, laboratory-bred adult *B. alexandrina* snails on colourless, transparent polythene sheets lining the inner walls of beakers (after 2 weeks of continuous ovipositioning), were used for the study. Egg masses were removed by cutting out small circles of polythene onto which they had attached and immediately examined with light microscopy to confirm viability Olivier and Haskins (1960) and extent of embryonation. Only viable eggs (Figure 1) were used for experimentation.

Four egg masses (each with 8 to 15 embryos) were exposed for 24 h, to 200 ml, each of seven different concentrations of an extract in 3 replicates. There were three replicates of control in which egg masses were exposed to dechlorinated tap water containing 0.5% DMSO. Egg masses were removed from the extracts at the end of the 24 h exposure period and thoroughly washed with dechlorinated tap water before incubation in beakers containing dechlorinated tap water at room temperature.

Individual embryos in an egg mass were microscopically examined weekly for development and hatching. Final assessment of mortalities was at the end of 4 weeks. An embryo in an egg mass was considered dead if its cells became opaque, dull or desegregated dos Santos et al. (2000) or if unhatched at the end of the experiment. Mortalities were recorded as the number and percentage of dead/ unhatched embryos.

Statistical calculation

The LC_{50} and LC_{90} values (with 95% confidence limits) of extracts for the egg masses were calculated by analysis of the mortality data and logarithm concentration, using a probit analysis computer software program (SPSS v. 10.0 for Windows).

RESULTS

The molluscicidal effect of ethanolic extracts of Fruits, leaves, roots and stem bark of *D. sissoo* on *B. alexandrina* snails after 24 hrs of exposure under the present laboratory conditions is presented in Table (1) and Figure (1). LC_{50} and LC_{90} values were 8.8 and 34.4 ppm for fruits, 12.5 and 64.3 ppm for leaves, 16.4 and 76.4 ppm for roots and 32.8 and 136.6 ppm for stem bark, respectively.

Toxicities of tested extracts of *D. sissoo*, as indicated by the percentage egg mortalities (percentage of dead unhatched embryos after four weeks of incubation) were concentration-dependent and increased with increasing concentration of extracts (Table 2).

Ethanolic extract of the fruits was the most active with 100% mortality at 50 mg/l, followed by those of the leaves (at 100 mg/l), roots (at 200 mg/l) and stem bark (at 400 mg/l) (Table 2). Their respective 24 hr-LC_{50} and LC_{90} values for *B. alexandrina* egg masses were 10.8 and 38.6 ppm, 18.5 and 68.3 ppm, 20.4 and 88.4 ppm, 36.8 and 144.6 ppm (Table 3). Extract toxicities were manifested as embryo deaths either at the gastrula/exogastrula (Figure 2) or pre-hatch snail (Figure 3) stage of development or both (Figure 4), depending on the concentration of extract. The percentage of embryo deaths at various stages increased with increasing concentration of extract. All eggs in the control groups hatched (Figure 5) by the second week of incubation.

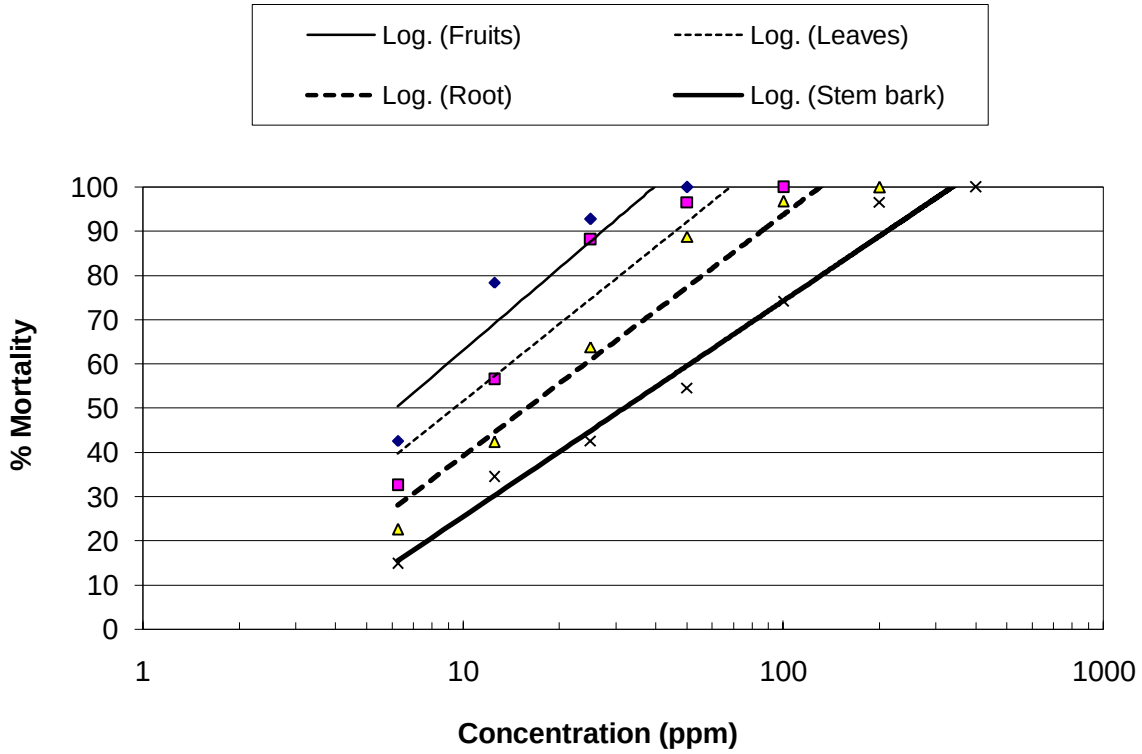

Figure 1. Molluscicidal activity of ethanolic extract of *D. sissoo* against *B. alexandrina* snails.

Table 1. Calculated lethal concentration values of ethanolic extract of *D. sissoo* for *B. alexandrina* snails.

Plant part	24 h LC_{50} (CI_{95})	24 h LC_{90} (CL_{95})
Fruits	8.8; 6.6-10.9	34.4; 30.6-38.4
Leaves	12.5; 10.6-14.8	64.3; 52.8-64.8
Roots	16.4; 12.4-20.6	76.4; 60.5-76.4
Stem bark	32.8; 20.8-32.5	136.6; 112.4-136.6

Table 2. Percentage of embryo deaths at pre-hatch snail stage of development four weeks post exposure.

Concentration (ppm)	Fruit	Leaf	Root	Stem bark
6.25	32.4 ± 3.1	22.5 ± 3.2	12.5±2.1	8.7 ±2.2
12.5	68.2 ± 6.2	48.4 ± 4.4	32.2 ± 3.8	23.3 ± 3.3
25	90.4 ± 4.8	68.2 ± 6.2	73.6 ± 4.6	48.2 ± 4.4
50	100	92.6 ± 6.5	80.8 ± 5.8	64.2 ± 4.3
100		100	92.8 ± 4.9	80.2 ± 6.0
200			100	94.6 ± 6.4
400				100

DISCUSSION

Results from this study have shown that the activity of a plant extract varies considerably according to the part of the plant (Kloos and McCullough, 1987; Sofowora, 1993). Ethanolic extract from the fruits was the most potent, followed by those from the leaves, roots and stem bark. Since active secondary metabolites do not always accumulate to the same degree in plant parts Duncan and Sturrock, (1987), Farnsworth et al. (1987), Lugt et al.

Table 3. Calculated lethal concentration values of ethanolic extract of *D. sissoo* for *B. alexandrina* egg masses.

Plant part	24 h LC_{50} (CI_{95})	24 h LC_{90} (CL_{95})
Fruit	10.8; 7.4-11.2	38.6; 32.4-40.8
Leaf	18.5; 14.6-22.8	68.3; 56.8-70.8
Root	20.4; 16.4-24.6	88.4; 66.5-92.4
Stem bark	36.8; 28.8-40.5	144.6; 122.4-166.6

CI_{95}= Confidence interval at 95%.

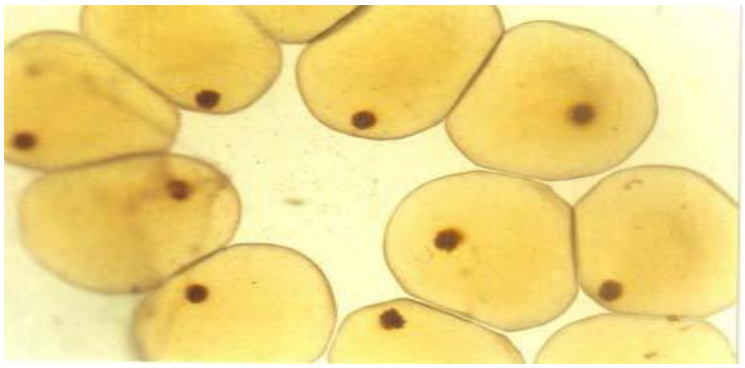

Figure 2. Photomicrograph of normal viable**,** 24 h–old egg mass of *B. alexandrina*. (×200).

Figure 3. Photomicrograph of dead embryos at the gastrula/exogastrula stage after 4 weeks of incubation. (×200).

(1987) their yield in a plant extract and consequently, the activity of the extract will vary considerably with the plant part. It would thus mean that in this study, the fruit extract had the highest yield of bioactive compounds. Since the yield of bioactive metabolites in a plant extract also varies considerably with the method/solvent of extraction Clark et al. (1997), Marston et al. (1993), it is plausible that the ethanolic extracts were generally more potent than the aqueous extracts probably because the active principles in the plant dissolved more readily in and were better extracted by a less polar solvent (ethanol) than water. This is in agreement with many literatures reporting of differences in the activities of extracts obtained from the same morphological part of a plant using different solvents. For instance, the methanolic extract of the fruits of *Tetrapleura tetraptera* is more potent than the aqueous extract (Adewunmi et al., 1982).

The viable 24 h old egg masses used in this study were susceptible in varying degrees, to the toxic action of the tested plant extracts. They were however, less susceptible than the adult snails for which the calculated LC_{50} values of the extracts were lower (Adenusi et al., 2008). Similarly, Ahmed and Ramzy, (1997) reported that the eggs of *B. alexandrina* were less susceptible to the

Figure 4. Photomicrograph of an egg mass containing dead embryos both at the gastrula/exogastrula and pre-hatch snail stages. (×200).

Figure 5. Photomicrograph of undeveloped eggs of *B. alexandrina* at the gastrula/ exogastrula stage after 4 weeks of incubation. (×200).

extracts of *Solanum nigrum* than the adult snails, on account of higher lethal doses. The higher lethal doses for embryonated eggs of other related *Biomphalaria* species, *B. pfeifferi* and *B. glabrata* compared to those for the adult snails have been reported in the literature for other products, including latex of the Crown of Christ, *Euphorbia splendens var. hislopii* Schall et al. (2001) and extracts from different parts of some species of *Ann* (dos Santos et al., 2001).

In the present study, the developmental stage of the embryos at which extracts manifested lethality was concentration-dependent. Majority of the eggs exposed to the lowest concentrations of extracts hatched, while those exposed to the highest concentrations were unhatched and contained dead embryos either at the gastrula/ exogastrula or pre-hatch snail stage of development or both. Hatching of eggs exposed to low concentrations of extracts could be due to the fact that these concentrations were not high enough to effectively interfere with normal embryonic development within the eggs, therefore their entry into them. Embryo deaths at the pre-hatch snail stage in eggs exposed to higher concentrations of extracts could have resulted from interference with the stages involved in the process of hatching.

Embryo death at the gastrula/exogastrula stage is indicative of acute extract toxicity, as such embryos never developed beyond the stages they were before exposure. This demonstrates the potency of the extracts at such concentrations, at arresting, within the 24 h exposure period, embryonic development beyond the gastrula/ exogastrula stage. Activity against both adult and egg stages of vector snails is considered one of the most important aspects of any efficient molluscicide that is to be used in the control of schistosomiasis (Farnsworth et al., 1987). In the present study, only the ethanolic extract of *D. sissoo* fruits could be considered as being reasonably active against *B. alexandrina* eggs (LC_{50} and LC_{90} values < 100 mg/l; 8.8 and 34.4 ppm for adult snails and 10.8, 38.6 ppm for egg masses). It is possible that multiple applications of the molluscicidal extract would probably be required to completely clear the snails from

schistosomiasis transmission sites, as some of the eggs would persist at molluscicidal concentrations, subsequently hatch and eventually develop to mature snails capable of continuing transmission of infection. Furthermore, the molluscicide will have to be applied before the newly hatched snails start to lay eggs.

Conclusion

The findings of this study have re-emphasized the need to explore the possibility of using allelochemicals derived from plants, particularly those used in alternative medicine practice, as supplementary and complementary measures in the control of schistosomiasis. As the overall efficacy of a molluscicide is greatly enhanced if it also shows significant toxicity towards snail eggs, an added advantage to the potential development of the ethanolic extract of *D. sissoo* fruits for use as a plant molluscicide is its lethality to the embryonated egg masses of *B. alexandrinai*. Even endod, the most promising plant molluscicide, is devoid of ovicidal properties (Lemma, 1970; Lemma and Yau, 1974). The practicability of the ethanolic extract of *D. sissoo* fruits in the control of schistosomiasis rests upon the fact that the fruit, being a regenerative plant part, does not require destructive harvesting, as would other plant parts.

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