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ARTICLE

Molluscicidal activities of ethanolic extracts of *Calotropis procera* and *Morinda lucida* against *Lymnaea natalensis*

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ABSTRACT Snails control is considered the most effective method in reducing the transmission of fascioliasis. Chemical molluscicides are reported to be toxic and ineffective due to snail resistance, hence, the need for safer and effective molluscicides. The aim of this study was to assess the molluscicidal activities of two medicinal plants against snail host of *Fasciola gigantica*. The molluscicidal activity of *Morinda lucida* and *Calotropis procera* was investigated against *Lymnaea natalensis*, the intermediate host of *F. gigantica*. Ethanolic extract of leaves of the tested plants were evaluated for their lethal activity against the embryos within the egg masses and adult *L. natalensis*. All the two tested plants exerted toxic lethal effect against both the embryos and the adult snails. While *M. lucida* extract appeared to be more potent at lower concentration on the embryos, the extract of *C. procera* leaves was significantly more potent than that of *M. lucida* in adult snails (P<0.05). The LC50 of ethanolic extracts of *M. lucida* and *C. procera* was 1.698 mg/ml and 0.500 mg/ml, respectively, at 72 h exposure time of adult *L. natalensis*. The two tested plants have molluscicidal activities, but these activities could be stage specific at certain concentrations.

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Introduction

Livestock play important role in human nutrition and socio-economic development as they are important sources of milk, meat, and eggs (FAO 2011). Animal health and productivity are often challenged by infectious diseases in developing countries (Ali et al. 2016; Elhaig et al. 2016). Fascioliasis, a zoonotic parasitic disease with heteroxenous life cycle is caused by *Fasciola hepatica* and *F. gigantica* and it is transmitted mostly by consumption of the infective stage of the parasites called metacercariae in water or vegetables such as watercress (Mas-Coma et al. 2005). The parasite develops in the definite host which could be either humans or herbivorous animals and inflicts significant tissue damage on them. The life cycle outside the definite host involves specific snail host, *Lymnaea* spp. which allows for intramolluscan development. Within the snails, the parasite transforms from miracidium to sporocyst which in turn develops into redia. Cercariae emerge from the later and escape from the snails into the surrounding. The cercariae attach to littoral plants in preparation for encystment into metacercariae.

Albendazole and tricalbedazole are the two main drugs of choice often administered against fascioliasis. However, there are increasing evidence of development of drug-resistant *Fasciola* spp. (Álvarez-Sánchez et al. 2006; Olaechea et al. 2011), thus, making chemotherapeutic management approach of fascioliasis inadequate. Since the snail intermediate hosts of *Fasciola* play important role in the parasite transmission cycle, the integration of snails control in the national control strategies of snail-borne trematode parasites could yield better results. This approach will break the parasite transmission cycle by eradicating the parasite obligate snail host. Among the most widely used chemical molluscicides is niclosamide. This commercially available chemical molluscicide has recorded success in the control of freshwater snails of medical importance but toxicity concerns against non-targeted organisms have necessitated a search for safer alternatives (Oyeyemi 2021).

Besides other biological agents like bacteria and fungi, plants have become potential sources of active agents with molluscicidal properties. Several plants materials extracted with different solvents have been reported to show molluscicidal activities (Adetunji and Salawu 2008; El-Sherbini et al. 2009; Salawu and Odaibo 2011; Mandefro et al. 2017). The advocacy for plant molluscicides is based on the premise that plant materials are safe and cost-effective compared to synthetic materials.

Morinda lucida and *Calotropis procera* are two plants with vast folkloric applications. The different parts of *M. lucida*

have long history in their folkloric use for the management of fever-related ailments, malaria, yellow fever, gonorrhea, jaundice, ulcers, piles, leprosy, and dysentery (Burkill 1985; Oliver-Bever 1986). The bioactive compounds in the root (damnacanthal and rubiadin-1-methylether) and in the leaf (ursolic acid) were reported to have antimalarial properties (Koumaglo et al. 1992; Cimanga et al. 2006). In folk medicine, *C. procera* has been used to treat several ailments including amoebic dysentery, cholera, epilepsy, rheumatism, cough, ringworm, malaria and intermittent fever, and others as compiled by Misra et al. (1993). Unlike *M. lucida*, few studies have studied the antiplasmodial activities of *C. procera* (Adejoh et al. 2021). *C. procera* was also tested on earthworm as a model organism for testing the antihelminthic potential of the plant (Shivkar and Kumar 2003). No literature has reported the potential of extracts from *M. lucida* and *C. procera* as molluscicidal agents. The aim of this study was therefore to evaluate the molluscicidal activities of these two medicinal plants. The study provided useful information on the possibility of deriving molluscicidal agents from these plant sources.

Materials and methods

Snail collection and identification

The freshwater snail *Lymnaea natalensis* were collected locally in Ondo City, from a freshwater pond formally used to culture fish. The snail was identified by an experienced malacologist in the Department of Biological Sciences, University of Medical Sciences, Ondo, Nigeria. The snails were allowed to acclimatize before they were used for the experimentation.

Plant collection and identification

Fresh leaves of *M. lucida* and *C. procera* were collected in April 2021 from the University of Ibadan premises and Ologuneru area in Ibadan, Nigeria, respectively. They were identified by a taxonomist in the Department of Botany, University of Ibadan, Ibadan, Nigeria, and voucher specimens (UIH-23073 and UIH-23074) were deposited at the herbarium. The leaves were washed under tap water to remove dirt and were then dried at room temperature for 21 days. The dried leaves were ground into fine powder and then used for the extraction process.

Extraction of bioactive components from plants

The extracts from the leaves were obtained using cold maceration method Nwofor et al. (2018). A total 140 g of *M. lucida* and 80 g of *C. procera* powdered leaves were macerated in 1.5 l and 1 l of 70% ethanol, respectively, at room temperature for 72 h. The mixtures were then agitated periodically to ensure complete extraction, after which they were strained using muslin cloth and filtered by Whatmann No. 1 filter paper. The extraction process was repeated two times with 70% ethanol. The extracts were then concentrated at 40 °C using a rotary evaporator under reduced pressure. Finally, the extracts were weighed and stored in a refrigerator till their usage.

Collection of egg masses and exposure to plant extracts

The egg masses were laid by the adult snails on a transparent polyethene bag that was used to line the bowl. The polyethene bag with attached egg masses was carefully cut out with a razor blade and then placed in a beaker containing distilled water, with the surface of the egg masses facing the water interface to enhance embryonic development and prevent dehydration. The egg masses were thereafter placed in different concentrations (2 mg/ml, 1 mg/ml, 0.5 mg/ml, and 0.25 mg/ml) of ethanolic extract of *M. lucida* and *C. procera*. The eggs were continuously exposed for 6 days. Embryos development was evaluated by photomicrography at ×40.

Molluscicidal testing

The adult snails collected were kept in a bowl of distilled water lined with transparent polythene bag to acclimatize to laboratory conditions for at least 24 h prior to the experiment. The snails were maintained with dried blanched lettuce. The working concentrations (2 mg/ml, 1 mg/ml, 0.5 mg/ml, and 0.25 mg/ml) were prepared from the two plants extracts. Adult *L. natalensis* (n = 5) were exposed to 5 ml of each of the working concentration and mortality was then monitor continuously for 72 h. The lethal concentrations (LC_{50}) which is the concentration required to kill half of the snails were determined. The negative control was 5 ml distilled water. Each exposure was performed in duplicate. Mortality was determined by observing for movement after suspected dead snails have been transferred to a different container containing distilled water.

Statistical analysis

Data on snail mortality were transferred to GraphPad Prism software (version 8.0) for analysis. Data were check for accuracy and then analyzed. One-way analysis of variance (ANOVA) was employed to test significant differences in the mortality of *L. natalensis* in different concentrations and exposure periods. Probit analysis was used to determine the LC_{50} of the two plants' extracts. *P*-values < 0.05 was considered statistically significant.

Results

There was no development beyond the blastula stage of

the embryos in the egg masses exposed to 2 mg/ml, 1 mg/ml, and 0.5 mg/ml of *M. lucida* and *C. procera* extracts (Fig. 1). While there was development of blastula into hippo stage in egg masses exposed to 0.25 mg/ml of *M. lucida* extract, the hippo stages were however deformed. The hippo stage in the egg masses exposed to 0.25 mg/ml of *C. procera* extract was normal as those in the negative control (Fig. 1H and I). A total of 86.2% of the hippo stage within the egg masses exposed to *C. procera* extract were normal and not ruptured (Fig. 2).

It was observed that the snails attempted to avoid the extract solution and exhibited some distress syndromes especially in higher extract concentrations. The snails exposed to 2 mg/ml of *M. oleifera* extract recorded 100% death after the 72 h, while all the snails exposed to lower concentrations survived (Table 1). Although weakness and lesser mobility were observed, no deaths were recorded in concentrations lesser than 2 mg/ml. The snails exposed to 2 mg/ml of *C. procera* extract were all recorded dead after the first 24 h, while those exposed to 1 mg/ml were all recorded dead after 72 h. A half (50%) of the snails exposed to 0.5 mg/ml concentration died after 72 h, while no snail was recorded dead in those exposed to 0.25 mg/ml of *C. procera* extract (Table 1). The LC_{50} of ethanolic extracts of *M. lucida* and *C. procera* was 1.698 mg/ml and 0.500 mg/ml, respectively, at 72 h exposure time of adult *L. natalensis* (Fig. 3).

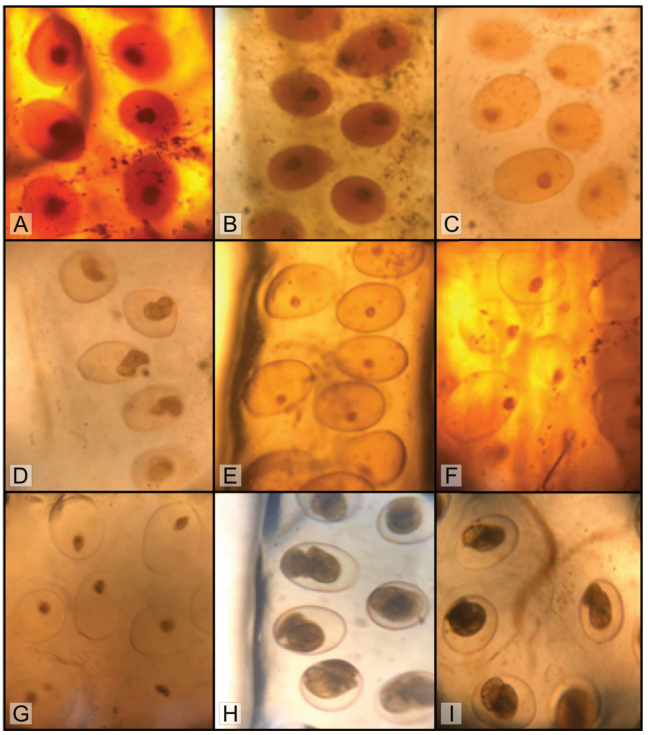

Figure 1. Ovicidal activity of *Morinda lucida* (ML) and *Calotropis procera* (CP) ethanolic extract. A: 2 mg/ml, B: 1 mg/ml, C: 0.5 mg/ml, D: 0.25 mg/ml (ML), E: 2 mg/ml, F: 1 mg/ml, G: 0.5 mg/ml, H: 0.25 mg/ml (CP), I: Control

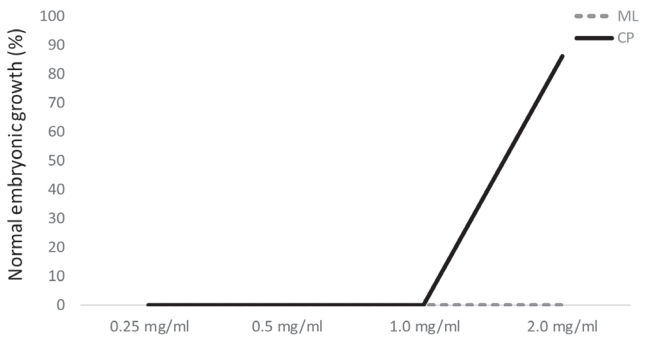

Figure 2. Percentage of normal embryonic growth in gelatinous egg mass after 6 days of treatment of plant extracts.

Discussion

Interrupting the transmission cycle of *Fasciola* through eradication of snails is considered one of the most effective integrated approaches for reducing the transmission and morbidity of liver fluke disease. As chemical molluscicides impose complex effects on man, domestic animals, fish and aquatic vegetation, there has been an increasing interest in alternative plant molluscicides (Bakry et al. 2011). The aim of this study was to test for the efficacy of *M. lucida* and *C. procera* as molluscicides for the control of snail intermediate host of fascioliasis.

The snails exhibited an irritative avoidance behavior in sub-lethal concentrations of plant extracts characterized by a continuous crawling out from the test solutions and aggregating at the water-air interface. This observation was like several previously reported studies (Adetunji and Salawu 2010; Salawu and Odaibo 2011; Omobhude et al. 2017). This behavior attempts to increase the survival rate and tolerance of snails in unfavorable environmental conditions. The survival of *Biomphalaria straminea* in sub-lethal doses of niclosamide was associated with extract-leaving behavior (Sarquis et al. 1997).

Comparison between the efficacy of the plant extracts against adult snails demonstrated that extracts of *C. procera* was more potent than *M. lucida*. This was also confirmed by the lower LC_{50} recorded in *C. procera* compared to *M. lucida* extracts. Lower LC_{50} indicates that lower concentration is capable to cause 50% in the snail mortality. *C. procera* is known to contain several cardioactive glycosides like calactin, calotoxin, usharin, usharidine, vouscharin, in addition to tannins, flavonoids, sterols and triterpenes which are likely to be responsible for the impressive molluscicidal activity in adult *L. natalensis* (Mossa et al. 1991).

Table 1. Mortality in *L. natalensis* exposed to plant extracts.

Concentration (mg/ml)	24 h		48 h		72 h		P-value
	ML	CP	ML	CP	ML	CP	
2	20.0	100.0	90.0	100.0	100.0	100.0	< 0.05
1	0.0	30.0	0.0	80.0	0.0	100.0	
0.5	0.0	0.0	0.0	10.0	0.0	50.0	
0.25	0.0	0.0	0.0	0.0	0.0	0.0	
Control	0.0	0.0	0.0	0.0	0.0	0.0	

ML – *Morinda lucida*; CP – *Calotropis procera*

In addition to the molluscicidal activities of the plants' extracts, the study showed that the two plants displayed ovicidal activity at higher concentration. This is an added advantage in the activities of the plants as snail controlling agents. The increase bioavailability of the active components of the plants extracts could be responsible for their higher ovicidal activity at higher concentrations. It is however important to note that *M. lucida* extract at lower concentration appeared to be more effective against the developing embryos as the hippo stage of the embryos were all ruptured compared to that of *C. procera* extract which assumed normal features after 6-day exposure of the egg masses. This observation negates what was observed in the adult snails and this is suggestive that activities of different plant molluscicides could be stage specific. In addition, although *M. lucida* extract may not necessarily induce adult snails' death, it could impair certain physiological processes in the snails. Curcuminnisin nanoparticle was reported to be weakly toxic to adult *Biomphalaria pfeifferi* but it caused significant reduction in egg-laying capacity in the snail (Omobhude et al. 2017).

Despite the potentials of these plants as molluscicidal agents or sources of molluscicidal agents, previous findings indicated that *C. procera* latex and ethanolic extract of leaves of the plant could induce marked cardiac and testicular toxicity like those of abamectin. The cardiotoxicity and testicular toxicity may be mediated by increased inflammatory response, oxidative stress, and suppression of antioxidant defense system (Ahmed et al. 2016). In a similar vein, a comprehensive review of the ethnobotanical uses of different parts of *M. lucida* by Adewole et al. (2021) also reported its potential teratogenic, mutagenic, genotoxic, hematotoxic, hepatotoxic or nephrotoxic effects.

In conclusion, *Calotropis procera* and *Morinda lucida* could be potential sources of plant molluscicides. The ethanolic extracts of these plants have wide spectrum of activities with significant effects on the adult snails and the developing embryos in the egg masses. Although plant-based agents are generally acclaimed safe and considered environmentally friendly, standardized methods to determine doses safe for human handling is strongly recommended as significant toxicity has been linked to many of them including those used in the study.

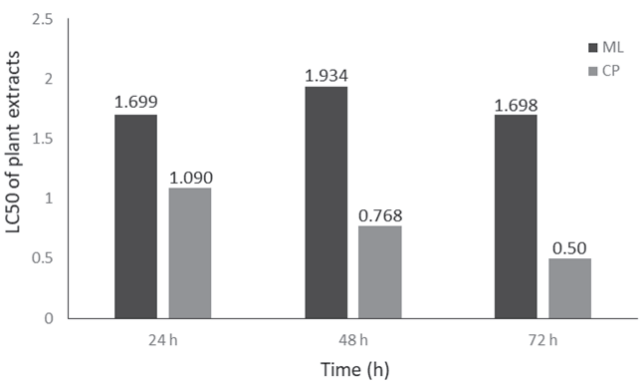

Figure 3. Variation in LC50 of *Morinda lucida* (ML) and *Calotropis procera* (CP) extracts with exposure time.

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