

Research Article

Bioactivity of *Areca catechu* seed extracts and *Azadirachta indica* seed oil against *Bulinus* sp., an intermediate host of *Schistosoma haematobium*

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ABSTRACT

Schistosomiasis is a parasitic disease transmitted by freshwater snails with infected *Schistosoma* parasites. The disease is endemic in many parts of Asia, Africa, and South America affecting people who cannot avoid contact with water, either because of their profession or because of a lack of reliable source of safe water for drinking, washing and bathing. Seed oil extract of *Azadirachta indica* as well as the aqueous and ethanol extracts of *Areca catechu* were tested on freshwater snails *Bulinus* sp. Bioassays were performed on eggs and adults of snails at varying concentrations (1, 2.5, 5, 7 and 10 mL/L for *A. indica* oil and 50, 100, 200, 400, 800 and 1600 mg/L for both the aqueous and ethanol extracts of *A. catechu*). The result revealed that mortality increased with ascending concentration. There was a significant difference in the molluscicidal activities of the three extracts ($P < 0.05$). Among the tested the plant extracts, *A. indica* seed oil showed the highest molluscicidal activity (100%) from 7 mL/L. Meanwhile, the lowest molluscicidal activity (2.5%) was found in the *A. catechu* aqueous extract. It was also observed that the potency of the extracts to inhibit egg hatching increased as the concentrations increased. *Azadirachta indica* seed oil and the ethanolic extract of *A. catechu* could be considered as a veritable means of controlling the schistosomiasis vector. Also, in endemic areas where communities are likely to accept the use of local plants, the studied plants stand as good candidates to replace the expensive conventional medicines.

Keywords: Schistosomiasis, *Bulinus* sp, *Azadirachta indica*, *Areca catechu*, efficacy.

RÉSUMÉ

La schistosomiase est une maladie parasitaire transmise à l'homme par un escargot d'eau douce porteur des schistosomes. La maladie est endémique dans de nombreuses régions d'Asie, d'Afrique et d'Amérique du Sud et touche les personnes qui ne peuvent éviter le contact avec l'eau, soit en raison de leur profession, soit en raison de l'absence d'une source fiable d'eau potable pour boire, se laver et se baigner. L'extrait d'huile de graines d'*Azadirachta indica* et les extraits aqueux et éthanoliques d'*Areca catechu* ont été testés sur des escargots d'eau douce *Bulinus* sp. Des bioessais ont été réalisés sur des œufs et des adultes d'escargots à différentes concentrations (1, 2,5, 5, 7 et 10 mL/L pour l'huile d'*A. indica* et 50, 100, 200, 400, 800 et 1600 mg/L pour les extraits aqueux et éthanol d'*A. catechu*). Les résultats ont révélé que la mortalité a augmenté avec la concentration. Il y a une différence significative dans les activités molluscicides des trois extraits ($P < 0,05$). L'huile de d'*A. indica* a montré l'activité molluscicide la plus élevée (100%) à partir de 7 mL/L. En revanche, l'extrait aqueux d'*A. catechu* a présenté l'activité molluscicide la plus faible (2,5%). Il a également été observé que la capacité des extraits à inhiber l'éclosion des œufs augmente avec les concentrations. L'huile d'*A. indica* et l'extrait éthanolique d'*A. catechu* pourraient être considérés comme de véritables moyens de lutte contre le vecteur de la schistosomiase. De plus, dans les zones endémiques où les communautés sont susceptibles d'accepter l'utilisation de plantes locales, les plantes étudiées pourraient remplacer les médicaments conventionnels qui sont coûteux.

Mots clés : Schistosomiase, *Bulinus* sp, *Azadirachta indica*, *Areca catechu*, efficacité.

1. INTRODUCTION

Schistosomiasis is caused by schistosomes which belong to the family Schistosomatidae, which includes species that are among the most dreaded parasites of humans (Schmidt & Roberts, 2000). Five clinically important species cause the majority of human infections (Cao *et al.* 2000). *Schistosoma mansoni*, *Schistosoma japonicum*,

Schistosoma mekongi, *Schistosoma intercalatum* and *Schistosoma haematobium*. Schistosomiasis is considered a neglected tropical disease and affects more than 250 million people in tropical and sub-tropical regions of the world, in poor communities without potable water or adequate sanitation. Sub-Saharan Africa accounts for approximately 90% of worldwide cases (WHO 2017). Disease assessments indicate that schistosomiasis accounts for up to seventy million disability-adjusted life years (DALY) lost annually, considering the amount of end-organ pathologies in the liver for *S. mansoni* and *S. japonicum* in the bladder and the kidney for *S. haematobium* coupled with chronic morbidities associated with impaired child growth and development, chronic inflammation, anaemia and other nutritional deficiencies (King & Dangerfield 2008). The freshwater planorbid snails; *Bulinus* sp. and *Biomphalaria* sp. are the intermediate hosts of *S. haematobium* and *S. mansoni*, which cause urinary and intestinal schistosomiasis respectively in Cameroon.

Snail control is one of the methods of choice for the fight against schistosomiasis transmission and might include the use of chemical molluscicides, bio-agents and environmental management. Chemical control depends on the elimination of snails from the habitat by molluscicides. Different molluscicides have been tested in the laboratory and in the field. They are either synthetically manufactured or of plant origin (Bustinduy *et al.* 2024). The synthetic molluscicide, niclosamide (Bayluscide®), is one of the most used chemicals because it fulfils most of the characteristics of the ideal molluscicide. Although it does not harm crop plants, it is slowly absorbed through the intestine, skin and mucous membranes of mammals. Amphibians and fish are, however, very sensitive to it because the chemical has a strong irritant effect on their mucosal membranes (Madsen 1985). Thus, an alternative control tool is desirable also because niclosamide has to be imported, it is expensive and has an environmental impact, even if small. Furthermore, applying only a single molluscicide such as niclosamide could possibly result in snails developing resistance to it (Madsen 1990).

Recently, molluscicidal plants have been drawing increasing attention for their environmental friendliness, accessibility, and cost-effectiveness. They are particularly suitable for community-based snail control activities in places where schistosomiasis transmission is more focal (Rollinson *et al.* 2013).

As the intermediate hosts, molluscs play a major role in the transmission of schistosomes and other pathogens like the liver fluke. They are the sites of an intense multiplication of parasites. Thus, snail control strategies are considered a priority for the reduction of schistosomiasis transmission. A standardized procedure devised for the laboratory screening of synthetic chemical molluscicides is used to evaluate plant molluscicides. Snail control by using synthetic molluscicides also plays an important role in the integrated control programme for schistosomiasis. Commercial molluscicides are not largely used as major control mechanisms due to their cost implications and toxicity effects on non-target organisms such as fish. Therefore, there is a need to search for cheap and safe local plants with molluscicidal properties (Massoud & Habid 2003). Much attention has been given to the study of plant molluscicides because they may provide a cheap, biodegradable and effective control approach in rural areas of developing countries, where schistosomiasis is endemic (Eze *et al.* 2020). However, the toxicity of these molluscicides to non-target organisms and ecosystem destruction may render them less efficient. The search for bioactive plants components which can be used as non-conventional molluscicides and antihelminthics has received considerable attention in recent times because of the increasing, worldwide development of resistance to chemical molluscicides in mollusc populations respectively. Jaiswal and Singh (2008) reported that ethanolic extract of *Areca catechu* seed from India is a potential source of botanical molluscicides against *Lymnaea acuminata*, a snail which is an intermediate host of *Fasciola hepatica*. However, in their study, Jaiswal and Singh (2008) used different solvent except the water. The molluscicidal property of *Azadirachta indica* from India against the snails *Lymnaea acuminata* and *Indoplanorbis exustus* was studied by Singh *et al.* (1996), where it was observed that the leaf, bark, cake, neem oil and the neem-based, pesticides, *achook* and nimbecidine were toxic to snails. It is known that the efficacy of botanicals varies with plant origin, the part of the plants used and the targets organisms (Nukenine *et al.* 2011). Therefore, scientific evidence to validate the use of plants of different origins is important. This is the basis on which the experiment was conducted using extracts of *A. indica* and *A. catechu* locally available in Cameroon to prevent schistosomiasis re-infection after treatment of humans.

2. MATERIALS AND METHODS

2.1. Collection and preparation of plant extracts

Mature seeds of *A. catechu* were locally collected based on ethnobotanical information. The plant seeds were collected around the University of Bamenda campus (N 5°59'22" and E 10°152'") and taken to the laboratory. The identified plant seeds at the Department of Plant Sciences of the University of Bamenda were air-dried and ground

into a fine powder using an electric blender, the powder was weighed and collected into clean cellophane bags, labelled and kept in a cool dry place until further use. Neem seed oil was obtained from Maroua, Far North region of Cameroon.

A quantity of 400 g of *A. catechu* powder was soaked in 2 L of 70% ethanol for two days (48 h), with constant shaking. The solution was filtered using filter paper (Whatman No. 1) and dried in a laboratory oven at 60°C. The dried materials constituted the ethanol extract (Das *et al.* 2010). Similarly, 400 g of *A. catechu* powder was soaked in 2 L of distilled water for two days (48 h) for polar compounds extraction. The solution was filtered using filter paper (Whatman N° 1) and dried as the previous extract. The dried material constituted the aqueous extract (Das *et al.* 2010).

2.2. Collection, sampling and breeding of snails

The snails used in this study were *Bulinus* sp. Six hundred adult snails of the genus *Bulinus* were collected from their natural habitat in the Bambui stream around the School of Agriculture of the University of Bamenda, kept in a clean aquarium and taken to the laboratory. In the laboratory, the snails were identified using the snail identification key of the Danish Bilharzias Laboratory (1983). In the laboratory, the snails were acclimatized for 72 h with dechlorinated tap water and maintained in aquaria of plastic bowls (12cm depth×30cm diameter with a capacity of about 5 L). Snails were fed with fresh lettuce after removing their midrib (WHO 1965). The aquaria were maintained with constant light for 12 h daily (12 h light and 12 h dark). Water was changed once a week or when necessary. Dead snails were removed as soon as possible from the troughs to prevent water fouling.

2.3. Preparation of egg-masses

Ten adult *Bulinus* snails taken from the stock aquaria were transferred to plastic trays (12 cm depth×30 cm diameter) containing 2 L of water. Then, 2-3 pieces of polythene sheets (about 5 cm× 15 cm) were placed in each plastic tray. The snails were fed with fresh lettuce and allowed to lay eggs. The polythene sheets were checked for egg masses. After this period the snails were transferred to a new plastic tray and the same steps were repeated. Polythene sheets containing the egg masses were easily located and isolated by cutting the plastic around each egg mass with a scalpel (about 0.5-1.0cm from the egg mass). The egg masses, each attached to a piece of polythene, were immersed three times in different Petri dishes containing clean water to remove any debris and transferred to containers containing 200 mL of dechlorinated tap water and the dishes were covered.

2.4. Bioassays

The molluscicidal evaluation was performed according to the WHO-recommended guidelines for molluscicidal tests (Gehad *et al.* 2009). Batches of 20 snails were pooled together in each clean 200 mL plastic container. The setups were left for 24 h and the snails were fed on fresh lettuce. Different concentrations of 1, 2.5, 5, 7 and 10 mL/L of *A. indica* oil extracts and 50, 100, 200, 400, 800 and 1600 mg/L of both the aqueous and the ethanol extracts of *A. catechu* were made. Tween 20 was used to mix water with neem seed oil. A control was set up with 100 mL of water only. After 24 h, the distilled water was discarded from the containers holding 20 snails each and replaced with 200 mL for the different extract concentrations, and the control preparation. Four replicates were set for each of the different concentrations made. The setups were left for 24 h and after this time the plant extracts were replaced with 100 mL water and mortality was recorded after an additional 24 h. This was done by poking the foot of the snail with a wooden applicator stick where lack of motion signified the death of the snail (Thomas & Asseffa 1979). The number of dead or surviving snails was recorded for each of the treatments and controls. The egg masses were cleaned and placed in groups of 10 in different plastic containers holding 100 mL of distilled water. The same procedure used for adult snails was applied and the egg masses were observed for hatching. Failure of egg masses to hatch within ten days signified mortality.

2.5. Statistical Analysis

Data on % corrected mortality and % egg inhibition were arcsine [(square root(x/100))] transformed and the number of eggs hatched were log (x + 1) transformed to homogenize the variance. The transformed data were subjected to the ANOVA procedure using the Statistical Analysis System (SAS). Tukey (HSD) test ($P = 0.05$) was applied for mean separation. Probit analysis was applied to determine the lethal concentrations causing 50% (LC_{50}) and 95% (LC_{95}) mortality of snails. Abbott's formula (1925) was used to correct for control mortality before Probit analysis and ANOVA.

3. RESULTS

3.1. Mortality of adult snails

The evaluation of the mortality of adult snails have revealed that mortality increased with increased concentration (Table 1). Aqueous extracts of *A. catechu* recorded an average percentage mortality of 37.5%, 35% and 70% for 400, 800 and 1600 mg/L respectively. Similarly, with the ethanol extract of *A. catechu*, it was observed that none of the snails died when they were exposed to 50 mg/L of the extract. Meanwhile, as the concentrations increased from 100 mg/L to 400 mg/L, mortality also increased from 12.5% to 17.5%. For 800 mg/L, 35% mortality was recorded, and the highest mortality was observed at 1600 mg/L (58.33%). *Azadirachta indica* extract exhibited varied degrees of mortality at different concentrations. 23.75% and 27.5% snail mortality were recorded at 1 mL/L and 2.5 mL/L respectively. Similarly, 85% mortality was recorded at 5mL/L. Meanwhile, 100% mortality was only achieved at 10 mL/L and 7 mL/L.

Table 1. Molluscicidal activity of *Areca catechu* and *Azadirachta indica* against *Bulinus* sp adult snails

Means percentage mortality of adult snails (% ± Standard error) #				
<i>Areca catechu</i> (means ± SE)			<i>Azadirachta indica</i> (means ± SE)	
Concentration mg/L	aqueous extract	ethanol extract	Concentration mL/L	Seed oil
0	0.00 ± 0.00 ^d	0.00 ± 0.00 ^d	0	0.00 ± 0.00 ^c
50	2.50 ± 1.44 ^{cd}	0.00 ± 0.00 ^d	1	23.75 ± 2.39 ^b
100	3.75 ± 2.39 ^{cd}	12.50 ± 1.44 ^{bc}	2.5	27.50 ± 1.44 ^b
200	6.25 ± 2.39 ^{cd}	12.50 ± 5.50 ^{bc}	5	85.00 ± 2.89 ^a
400	37.50 ± 3.54 ^b	17.50 ± 1.44 ^b	7	100 ± 0.00 ^a
800	35.00 ± 2.89 ^{bc}	35.00 ± 2.89 ^{ab}	10	100 ± 0.00 ^a
1600	70.00 ± 2.04 ^a	58.33 ± 1600 ^a		
F _(6; 21)	49.03 ^{***}	187.10 ^{***}	F _(5; 18)	911.48 ^{***}

#Each value is a mean ± standard error of four replicates. Means followed by the same upper-case letter along the column do not differ significantly at $P=0.05$ (Tukey's test), *** $P < 0.001$.

3.2. Mortality of egg masses

Table 2. Molluscicidal activity of *A. catechu* aqueous extract against egg masses of *Bulinus* sp.

Means number hatched eggs and percentage inhibition of egg (% ± Standard error) #				
Concentration mg/L	Aqueous extract		Ethanol extract	
	Number of hatched eggs	% of egg inhibition	Number of hatched eggs	% of egg inhibition
0	20.00 ± 0.00 ^a	0.00 ± 0.00 ^c	20.00 ± 0.00 ^a	0.00 ± 0.00 ^c
50	23.75 ± 0.25 ^b	76.25 ± 1.25 ^b	32.50 ± 0.29 ^b	67.50 ± 1.44 ^b
100	11.25 ± 0.25 ^c	88.75 ± 1.25 ^a	17.50 ± 0.29 ^c	82.50 ± 1.44 ^a
200	1.25 ± 0.25 ^d	98.75 ± 1.25 ^a	5.00 ± 0.58 ^d	95.00 ± 2.89 ^a
400	0.00 ± 0.00 ^d	100 ± 0.00 ^a	0.00 ± 0.00 ^d	100 ± 0.00 ^a
800	0.00 ± 0.00 ^d	100 ± 0.00 ^a	0.00 ± 0.00 ^d	100 ± 0.00 ^a
1600	0.00 ± 0.00 ^d	100 ± 0.00 ^a	0.00 ± 0.00 ^d	100 ± 0.00 ^a
F _(6; 21)	2001.1 ^{***}	610.42 ^{***}	742.50 ^{***}	246.72 ^{***}

#Each value is a mean ± standard error of four replicates. Means followed by the same letter along the column do not differ significantly at $P=0.05$ (Tukey's test) *** $P < 0.001$

Aqueous extracts of *A. catechu* recorded an average percentage mortality of 76.25%, 88.75% and 98.75% for 50, 100 and 200 mg/L respectively (Table 2). Similarly, the ethanol extract of *A. catechu* recorded an average percentage mortality of 67.5%, 82.5% and 95% for 50, 100 and 200 mg/L respectively (Table 2). In all the tested extracts, *A. indica* seed oil had the highest percentage mortality against the egg masses achieving 91.25% mortality

at only 1mL/L (Table 3). Complete egg hatch inhibition (100%) is recorded with *A. indica* seed oil from the content of 2.5 mL/L while both *A. catechu* extracts got similar results at the concentration of 400 mg/L.

Table 3. Molluscicidal activity of *A. indica* seed oil against *Bulinus* sp. egg masses

Concentration mL/L	Number of hatched eggs (mean ± SE) [#]	% of egg inhibition development (mean ± SE) [#]
0	20.00 ± 0.00 ^a	0.00 ± 0.00 ^b
1	8.75 ± 0.25 ^b	91.25 ± 1.25 ^a
2.5	0.00 ± 0.00 ^c	100 ± 0.00 ^a
5	0.00 ± 0.00 ^c	100 ± 0.00 ^a
7	0.00 ± 0.00 ^c	100 ± 0.00 ^a
10	0.00 ± 0.00 ^c	100 ± 0.00 ^a
F _(5; 18)	6225.00 ^{***}	4092.56 ^{***}

[#]Each value is a mean ± standard error of four replicates. Means followed by the same letter along the column do not differ significantly at $P= 0.05$ (Tukey's test); ^{***} $P < 0.001$

3.3. Toxicity of plant extracts

The evaluation of the plant extracts toxicity on adults *Bulinus* sp. revealed that the lethal concentration that killed 50% (LC₅₀) of the adult *Bulinus* sp. snails, when *A. indica* seed oil, aqueous and ethanol extracts of *A. catechu* was used were: 2.20 mL/L, 0.93 mg/L and 1.35 mg/L, respectively, while the lethal concentration that killed 95% of the snails (LC₉₅) were: 7.67 mL/L, 7.97 mg/L and 21.24 mg/L, respectively (Table 4). The toxicity of plant extracts on eggs showed that the lethal concentration that reduced 50% (LC₅₀) of the egg masses hatching when *A. indica* extract, aqueous and ethanol extracts of *A. catechu* were applied are: 0.80 mL/L, 0.03 mg/L and 0.03 mg/L, respectively, and the lethal concentration that inhibited the hatching of 95% of the egg masses (LC₉₅) were: 1.04 mL/L, 0.13 mg/L and 0.18 mg/L, respectively (Table 5).

Table 4. Toxicity of the plant extracts *Bulinus* adult snails

Products	Slope ± SE ^a	R ²	LC ₅₀ (95% FL ^b)	LC ₉₅ (95% FL)	X ² (p-value)
<i>A. catechu</i> aqueous	1.76 ± 0.30	0.77	0.93 (0.58 - 2.20)	7.97 (3.27 - 119.06)	16.45 (0.02)
<i>A. catechu</i> ethanol	1.37 ± 0.25	0.78	1.35 (0.76 - 5.09)	21.24 (5.46 - 118.28)	12.99 (0.01)
<i>A. indica</i> seed oil	2.20 ± 0.95	0.93	2.20 (101.31 - 1545)	7.67 (3.71 - 137.94)	56.61(0.01)

^aSE= Standard Error; ^bFL = Fiducial limit.

Table 5. Toxicity of the plant extracts on the egg masses

Products	Slope ± SE ^a	R ²	LC ₅₀ (95% FL ^b)	LC ₉₅ (95% FL)	X ² (p-value)
<i>A. catechu</i> aqueous	2.39 ± 0.42	0.71	0.03 (0.01 - 0.04)	0.13 (0.11 - 0.19)	1.83 (0.03)
<i>A. catechu</i> ethanol	2.22 ± 0.31	0.78	0.03 (0.02 - 0.04)	0.18 (0.15 - 0.26)	2.05 (0.01)
<i>A. indica</i> seed oil	14.78 ± 0.78	0.61	0.80 (0.42 - 2.31)	1.04 (0.92 - 3.02)	0.99 (0.1)

^aSE= Standard Error; ^bFL = Fiducial limit.

4. DISCUSSION

It was apparent from this study that the seed oil extract of *A. indica* and the aqueous and ethanol extracts of *A. catechu* possess molluscicidal properties against *Bulinus* sp. adults, which increased with the rising concentration. Neem seed oil was highly toxic to freshwater adult snails compared to *A. catechu* extracts. Lower concentrations (1, 2.5 and 5 mL/L) did not give 100% mortality, indicating that the snails could withstand the toxicity of the extracts at lower concentrations. Reduced mortality observed at lower concentrations may be due to low

concentration and the time frame, while higher mortality observed at higher concentrations within a short time can probably be linked to the oil toxicity and concentration itself. These results are in accordance with previous findings which reported the ascending efficacy of plant extracts depends on the concentrations applied (Jaiswal & Singh 2018; Pereira et al. 2020, Han et al. 2024). Botanical affect snails in different ways depending on the physiological characteristics of the snail species as well as the type of the molluscicidal plant (Ibrahim & Ghoname 2018). The mortality caused by *A. catechu* seed extract might be due to the presence of an alkaloid arecoline hydrobromide (Jaiswal & Singh 2008). It is therefore important to carry out chemical studies to determine and quantify the compounds found in the *A. catechu* of the present work and to study the mode of action of the extracts on adult *Bulinus* snails. Neem seed contains azadirachtin which is the main active ingredient to possess molluscicidal activity and is known to cause paralysis of the pest mouth, reduce respiratory activities and limit oxygen intake, therefore, leading to a slow death of the snails; it also has anti-appetent activities contributing to the snail mortality with time (Singh et al. 1996, Tofel et al 2017).

Plant materials may release active ingredients slowly so that the effect on the snail population is delayed, that is, the plant may act as a slow-release matrix. In this mode, there may also be effects on feeding and oviposition. Other plant substances affect orientation and feeding behaviour (Hoste *et al.* 2008). A bioassay of whole plants or parts in which snails are killed within 24 hours at a dosage below 100 mg/L indicates that the molluscicide is released quickly and the material may be a good candidate as molluscicide (Gordan, 1983). This agrees with the findings of this study in which the death of snails was observed within 24 hours of exposure to concentrations below 100mg/L of the plant extracts.

Snails exposed to *A. indica* exhibited behaviours that suggested they had been adversely affected by these plants. The snails were weak and could neither eat nor retract into their shells. They exhibited excessive mucus secretion and cessation of feeding. Increased mucus production followed by increased mucus secretion as observed in this study, is one of the first reactions of gastropods to many stressors, including mechanical stimuli or chemical irritation caused by molluscicidal chemicals (Triebkorn & Ebert 1989, Triebkorn *et al.* 1998, Port & Port 1986). One effect of the extruded mucus is to form a protective barrier preventing direct contact between the toxin and the epithelia of the skin or digestive tract, to reduce the toxicity of the chemicals (Port & Port 1986).

No progeny emerged when the *A. indica* seed was applied on eggs, except the lowest content. As the oil compounds can infiltrate the egg shield, some quantity of azadirachtin and other compounds like nimbin and salanin in neem oil may have entered the egg and prevented the eggs from hatching. These compounds have growth regulatory effects on eggs, such as growth inhibition, and malformation (Isman, 2006), which may have blocked the production of adult snails. The mechanism by which action *A. catechu* inhibited the hatchability of snail eggs is unknown and therefore further studies are necessary to clarify that activity.

The LC_{50} of the extracts was determined to measure the dose that killed or immobilised 50% of the target organisms within the treatment period. LC_{50} figures are frequently used as a general indicator of a substance's acute toxicity (Walker & Lupien 2000). A large LC_{50} means it takes a large quantity of the material to cause a toxic response (Fleming & Hunt 2000). $LC_{50} < 100$ mg/L indicates that the substance is highly toxic, $LC_{50} > 100 < 500$ mg/L indicates that the substance is moderately toxic, $LC_{50} > 500 < 1000$ mg/L indicates that the substance is weakly toxic while $LC_{50} > 1000$ mg/L indicates that the substance is non-toxic (Nguta et al. 2013). Among the extracts used on adult snails, *A. indica* oil extract was highly toxic on adult snails; $LC_{50} < 100$ mg/L. *Areca catechu* aqueous extract was weakly toxic; $LC_{50} > 100 < 500$ mg/L while *A. catechu* ethanol extract was nontoxic on the adult snails; $LC_{50} > 1000$ mg/L. When these extracts were tested on the egg masses, they were all highly toxic with *A. indica* being efficacious. For a plant to be considered a molluscicide, it should be registered in concentrations of up to 100 mg/L (Mott 1987).

5. CONCLUSION

Freshwater snails cause enormous problems in endemic areas and that inadequate control can lead to serious problems affecting inhabitants of such areas. Existing control measures are not enough to deal with the emergence or outbreaks of diseases caused by freshwater snails. Therefore, continued research including using plant-based products is important to produce botanical molluscicides that are cheap, less toxic and effective to control freshwater snail population. The intermediate hosts play an essential role in the parasite life cycle. Molluscicides are therefore very crucial for controlling schistosomiasis if appropriately used. This research shows that the seed oil extract of *A. indica* had a high molluscicidal effect on *Bulinus* sp while the aqueous and ethanol extract of *A. catechu* were less toxic, and the extracts had an inhibitory effect on the development of the egg masses. For this

reason, the use of plant molluscicides may be one of the veritable means of controlling schistosomiasis and other trematode infections. In addition, endemic communities are likely to accept the use of local indigenous plants since they are familiar with their properties and growth characteristics.

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