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A PRELIMINARY STUDY ON THE MOLLUSCICIDAL ACTIVITIES OF SOME SELECTED INDIGENOUS PLANTS AGAINST SCHISTOSOMOSIS INTERMIDIATE HOSTS *(BULINUS* AND *BIOMPHALARIA SPP)*

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ABSTRACT

The molluscicidal activities of methanolic extracts of *Acanthospermum hispidium, Ricinus cuminis* and *Vernonia amygdalina* leaf and root against *Biomphalaria pfeifferi* and *Bulinus globosus* were investigated. Ten adults each of *Biomphalaria pfeifferi* and *Bulinus globosus* were exposed to different concentrations; 40, 80, 120, 160 and 200ppm of leaf and root extracts respectively. The snails were recovered after 24 hours of exposure. The lethal dose (LD_{50}) of the three plants against *Biomphalaria pfeifferi* and *Bulinus globosus* were 34.24, 12.27 and 25.96ppm; and 43.66, 10.55 and 37.30ppm for leaf and root extracts respectively. The methanolic extracts of the three plants were very potent against *B. globosus* and *B. pfeifferi* at (x^2=3.78, p<0.05), (x^2= 3.03, p< 0.05), and (x^2 = 3.37, p<0.05) for *Acanthospermum hispidium, Ricinus cuminis* and *Vernonia amygdalina* leaf extracts, and ($x^2$4.50, p<0.05), (x^2= 2.52, p<0.05) and (x^2 = 3.23, p<0.05) for root extracts. The results obtained showed that the leaf and root extracts of the three plants possess molluscicidal properties against the snails therefore they are suitable for biological application which offers a potentially simple, readily available, inexpensive and environmentally safe molluscicidal agents of plant origin for controlling urinary and intestinal schistosomosis and deserves further studies in order to identify and characterize its molluscicidal components; and to ascertain their effectiveness on the field.

KEYWORDS: Molluscicides, *Vernonia amygdalina, Acanthospermum hispidium, Ricinus cuminis, B. globosus, B. pfeifferi.*

INTRODUCTION

Schistosomosis remains one of the most prevalent parasitic infections in the world. It is endemic in 78 countries and territories and continues to be a global public health concern in the developing world (Fenwick, 2012; Gryseel *et al*., 2006; WHO, 2014). In Nigeria, the disease is also considered to be a public health problem particularly among rural and sub-urban areas. (Stohard and Russell, 2005). Schistosomosis is widespread in Nigeria in both rural and urban settlement (Ugbomoiko *et al*., 2010), which is caused by endemic parasites of the genus *Schistosoma,* in tropical and subtropical regions. Schistosomosis is contracted when persons come in contact with infected water that habours the parasites (Bassey, 2011). Because it is a chronic insidious disease, it is poorly recognized at early stages, and becomes a threat to development by disabling men and women during their most productive years (WHO, 2016). Although the distribution of schistosomosis have changed over the past 50 years, and there have been successful control programmes, the number of people estimated to be infected or at risk of infection remains unchanged (WHO, 2016).

Despite major advances in control and substantial decrease in morbidity and mortality, schistosomosis continues to spread to new geographic areas. Environmental changes that result from development of water resources and the growth and migration of population can facilitate the spread of schistosomosis (Kheir *et al.*, 1999). Chemotherapy of schistosomosis only provides momentary abatement of human parasites burden because of rapid re-infection rates after treatment (Agboola *et al.*, 2011).

One of the water-based approaches to controlling schistosomosis is to interrupt the life cycle by interventions that impact the intermediate host. This has most commonly been done through the use of chemical molluscicides such as Niclosamide (Yang *et al*., 2012). The drawback of this approach, however, is that the

chemicals that kill snails are nonspecific and are toxic for other aquatic animals such as fish, which often make up the protein source for persons in communities where schistosomosis is endemic. Fish toxicity and yellowing of treated water by niclosamide decrease the acceptability of mollusciciding these in communities (Takougang *et al.*, 2007). In addition, the chemicals are expensive and can be rapidly washed down streams following rains or diluted to nontoxic concentrations in larger water bodies, which necessitates frequent reapplication. Furthermore, a relatively high degree of training is needed for personnel who disburse molluscicides as efficacy is influenced by other environmental conditions such as water hardness and temperature. Indigenous plant extracts are an attractive alternative to chemicals for killing snails. Costs are held down by local availability and the extracts are less toxic to other forms of aquatic life (Mølgaard, 2000). It is therefore justifiable and very important to screen and evaluate the potency of some local plants on the snail intermediate host of Schistosomosis, especially in rural communities where these plants abound.

Plant extracts have found application as molluscicides due to certain metabolites they possess, and are evolving as a cheap and bioavailable molluscicide in schistosomosis control; as such it is necessary to evaluate molluscicidal activities of methanolic extracts of leaves and roots of selected Nigerian plants against *Biomphalaria pfeifferi* and *Bulinus globosus. Ricinus cuminis, Acanthospermum hispidum and Vernonia amygdalina,* has been identified as being used traditionally in the treatment of schistosomosis in some parts of the Federal Capital Territory (Abdulrahim *et al.* 2008), hence the need to evaluate their molluscicidal activities.

MATERIALS AND METHODS

3.1 Study Area

Gwagwalada is a town in the Abuja Capital Territory region of Nigeria. It is one of the six Area Council Headquarters of Federal Capital Territory. Gwagwalada is about 45km away from Abuja city. It lies between 8^0 55' and 9^0 00N and longitude $7^0$00 and $7^0$05E.Gwagwalada has tropical humid hot climate with two seasons. The rainy season lasts from April to October, followed by dry season from November to march. (Ishaya*et al*. 2010).

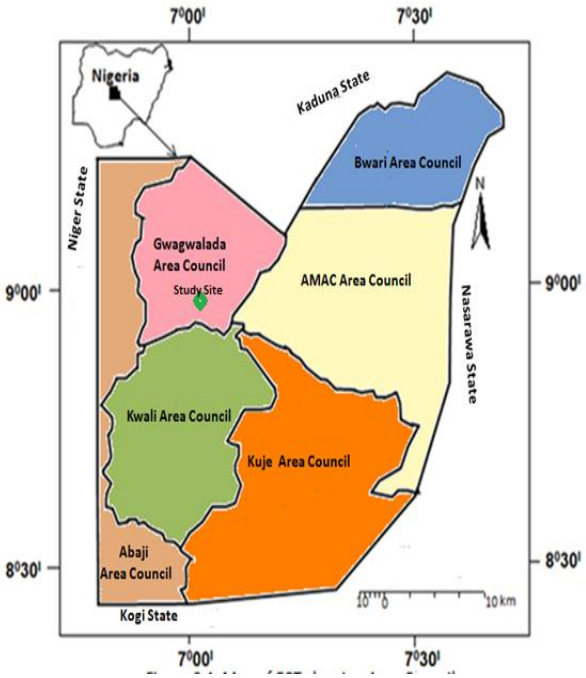

Fig. 3.1: Map of Federal Capital Territory, Showing the Study Site.

Collection of Plants

Acanthospermum hispidium, *Ricinus cuminis* and *Vernonia amygdalina* were harvested in the month of September, 2015 and separated into morphological parts (leaves, and roots) and dried in the shade. Voucher specimen were kept in the herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu.

Preparation of Plant Extract

Methanolic extraction of dried materials of the selected plants was carried out as described by Tariwari *et al.* (2014) with slight modification using 70% methanol. Shade-dried leaves, and roots of the collected plants were pounded with mortar and pestle and macerated in 500ml of 70% methanol for 72 hours and thereafter the solvents was concentrated to dryness using a rotary evaporator at 65°C leaving no trace of methanol. The dried concentrated extracts were stored in a specimen bottles and kept at 4^0C until it is needed.

Phytochemical Screening

Prior to the bioassay, the extracts of the plant were subjected to phytochemical screening, using standard procedure of Ahirrao *et al*. (2011), in order to detect some of the phytochemicals present.

Collection of Snails

A total of six hundred and fifty *Bulinus globosus* and *Biomphalaria pfeifferi* were collected from a slow moving stream in Gwagwalada River along Paikon Kore in FCT Abuja, using steel-framed sampling net as well as manual picking with forceps, in the month of October, 2015. The collected snails were transferred to a sterile plastic container, containing water from the river and

taken to the Parasitology Laboratory, Faculty of Veterinary Medicine, University of Abuja for identification.

Laboratory Maintenance of the Snail

The collected snails were put in sterile plastic containers (15cm depth×30cm diameter with a 5 litre capacity), containing some of the water from the river, where the snails were collected. The plastic containers were designed to suit their natural habitat with stick, sand, and some stones. They were fed with lettuce (salad leaves) and left for four weeks to acclimatize to laboratory conditions before the commencement of the *in vitro* assay (Otarigbo and Morenike, 2012).

Molluscicidal Potency Test of Plant Extracts on the Snails.

The molluscicidal potency test was carried out according to the standard method prescribed by WHO, (1983). For each plant extracts, five (5) different concentrations 40, 80, 120, 1600 and 200ppm were prepared from the dried methanolic extract of each plant parts using equal volume of 500ml rain water in a beaker (6 cm depth×11cm diameter). A minimum of 10 snails per test container (beaker) were placed in the different concentrations of the extracts, and observed every 6 hours for the number of snails crawling upwards. In each set up, the snails were prevented from crawling out of the trough by means of a fine mesh cloth tied to the beaker by rubber band. The snails were not fed during the course of the experiment, it had been observed that healthy snails live up to 5days or more without food, provided other environmental conditions are constant (Adetunji and Salawu, 2010). Rain water was used as untreated control.

Recovery of the snails

After 24 hours of exposure to the plant extracts, the snails were transferred to fresh rain water and maintained there for another 24 hours. Death of the snails was determined and confirmed by the lack of reaction to irritation of the foot with a blunt wooden probe to elicit typical withdrawal. Thereafter, mortality counts were recorded.

Statistical Analysis.

The data was subjected to the probit analysis using Chi-square and regression coefficient. The lethal concentration that killed 50% of the snails (LD_{50}) was determined using the SPSS version 20.

RESULTS

The results of the tested plant extracts, screened against *B. globosus* and *B. pfeifferi*, was carried out based on methods of determining plant efficacy, according to standards required for the screening of molluscicide (WHO, 1983).

The phytochemical screening of the methanolic extract of the plants indicates the presence of Tannins and Saponins in the leaf extract, while there is presence of Saponins, Tannins and Flavonoids in the root extract of *Acanthospermum hispidium.* The result also showed the presence of Saponins in both the leaf and the root extracts of *Ricinus cuminis.* There is presence of Saponins and Tannins in the leaf extracts of *Vernonia amygdalina*, with only Saponins in the root extracts of the same plant. It is well documented in literature that, there are over 25,000 terpenes identified from plants; Tannins, Saponins and Flavonoids are examples of Terpene and are said to exhibit potential molluscicidal properties, especially those of the Saponins, because of their ability to affect surface tension due to their froth-forming ability (Tariwari, 2014).

Table 1: Results of the Phytochemical Screening of Methanolic Extracts of *Acanthospermum hispidium, Ricinus cuminis* and *Vernonia amygdalina.*

Phytochemicals	***Acanthospermumhispidium***	***Ricinus cuminis***	***Vernonia amygdalina***
Alkaloids	_	_	_
Saponins	+	+	+
Tannins	+	_	+
Flavonoids	-	_	
Terpenoids	_	_	

Positive (+) =present
Negative (-) =absent

In this study, the methanolic extracts of leaf of *Ricinus cuminis, Acanthospermum hispidium* and *Vernonia amygdalina* were evaluated for molluscicidal activity against *B. globosus* and *B. pfeifferi snails.*

The present data revealed that methanolic extract of *Acanthospermum hispidium* leaves showed the highest molluscicidal activity (LD^{50} =12.27), followed by *Vernonia amygdalina* (LD^{50}=25.96) and *Ricinus cuminis* (LD^{50} = 34.24). The methanolic leaf extracts of the three plants were very potent against *B. globosus* and *B. pfeifferi* at (x^2=3.78, p<0.05) for *Ricinus cuminis*, (x^2= 3.03, p< 0.05) for *Acanthospermum hispidium*, (x^2 = 3.37, p<0.05) for *Vernonia amygdalina*. There were strong positive correlation between mortalities observed in both *B. globosus* and *B. pfeifferi* and the methanolic

extract concentrations of the *Ricinus cuminis, Acanthospermum hispidium* and *Vernonia amygdalina* leaves (Table 2).

Table 2: Statistical Analysis of Molluscicidal Effect of Methanolic Extract *of Ricinuscuminis, Acanthospermumhispidium and Vernoniaamygdalina*leaf on *Bulinusglobosus*and *Biomphalariapfeifferi.*

LEAF	Regression Eqn	Chi-Square (p<0.05)	*Bulinus globosus* (mean)	*Biomphalaria pfeifferi* (mean)	LD_{50}
Ricinus cuminis	Y=1.647-0.0481x	3.776	2.5	2.5	34.24
Acanthospermum hispidium	Y=0.341-0.0278x	3.031	4.50	4.83	12.27
Vernonia amygdalina	Y=1.298-0.050x	3.371	4.167	4.33	25.96

The statistical analysis also showed that there is no significant difference in the mean morbidity between *B. globosus* and *B. pfeifferi* in all the extracts used (Tables 2). This suggests that the molluscicidal potency of the extracts used is independent of the different species of the snails.

DISCUSSION

This study revealed that adult *B. globosus* and *B. pfeifferi* were susceptible to leafextracts of these plants at different concentrations. The withdrawal of each snail in the untreated (control) water in the containers into their shell and their subsequent activity after a few minutes was also reported in Egypt by El-Sherbini *et al.,* (2009). This observation may be due to slight changes in the physiochemical conditions of the water in the containers. At the introduction of the extracts, the snails withdrew into their shell again, however they started crawling out of the water, most staying at the water-air interface with their shell partially immersed in the water. Similar observation was made by Adetunji and Salawu (2010). The mechanism of *B. globosus* and *B. pfeifferi* partially leaving the treated water has been found to increase the survival of snails (Otarigbo and Morenike, 2012).

The potency of the extracts in the present study were much higher when compared with results from other works. For instance Otarigbo and Morenike (2012), reported that ethanolic leaf extract *of Chromolaen aodorata* on the adult *B. pfeifferi* had LD_{50} as 88.04ppm. In another research, Otarigbo and Morenike (2012), reported that the LD_{50} of ethanolic leaf extract of *Cymbopogon citratus* on the adult *B. pfeifferi* was 61.79ppm. Hatil *et al.* (2010) had similar results when they used aqueous extract of *Cymbopogon nervatus* leaves on *B. pfeifferi*, they had LD_{50} of 213.099ppm and LD_{95} of 506.69ppm. They also used the same aqueous extract of *Cymbopogon nervatus* leaves on *Bulinus truncatus* and had LD_{50} and LD_{90} of 237.33ppm and 331.05ppm.

Chi Square (x^2) analysis showed that molluscicidal potency of all the plant extracts used showed significant mortality rates of *B. globosus* and *B. pfeifferi* ($P<0.05$) at different concentrations. The correlation coefficient in all cases showed that there were strong positive correlations between mortalities observed in *B. globosus* and *B. pfeifferi* and extract concentrations of the plants used. It was also observed in this study that there was no significant difference in the *mean* morbidity between *B. globosus* and *B. pfeifferi*. This is contrary to the observations of Tariwari *et al.* (2014). In this study, methanol was selected for extraction. It is well documented in literatures that compounds from plants which possess molluscicidal properties are well enriched in methanolic extracts ((Rug and Ruppel, 2000; Devappa *et al.*, 2010; Bassey *et al*., 2013; Angaye, 2013; Tariwari *et al*., 2014). Furthermore, in this research, it is worthy of note that during the screening, all snails died within 12 hours, which meets WHO standards of less than 24 hours for an active molluscicide.

CONCLUSION

The results of the present study have shown that *Ricinus cuminis, Acanthospermum hispidium* and *Vernonia amygdalina* possesses molluscicidal properties against the snails *B. globosus*and *B. pfeifferi* and therefore they are suitable for biological application which offers a potentially simple, readily available, inexpensive and environmentally safe molluscicidal agents of plant origin for controlling urinary and intestinal schistosomosis. Use of plant molluscicides not only may eliminate the economic burden of importing expensive synthetic molluscicide but also could stimulate growth of small-scale industries in developing countries. If plant molluscicides are to be applied successfully and in long-term, they will sustain the control of schistosomosis.

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