Molluscicidal Effect of Vernonia Amygdalina (Del) and Momordica Charantia. Linn. on Bulinus (Phy) Globosus

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Abstract*— The molluscicidal activities of aqueous, methanolic and ethanolic extracts of *V.amygdalina* and *M.charantia* leaves against the juvenile and adult *Bulinus* (Phy) *globosus* were carried out. Five juveniles and five adults each were exposed to different concentrations of 200ppm, 400ppm, 600ppm and 800ppm of aqueous, methanolic and ethanolic extracts of the two plants. Exposure period of 24hours was observed for both juvenile and adult snails. The mean lethal concentration LC50 that killed 50% of the juveniles using aqueous, methanolic and ethanolic extract of *V.amygdalina* were (534.05ppm, 182.86ppm, 208ppm) and (558.99ppm, 269.86ppm and 236.9ppm) for *M.charantia*. The corresponding values for LC_{90} of *V.amygdalina* were (729.83ppm, 414.89ppm and424.27ppm) and (622.63ppm, 419.73ppm and1029.48ppm) for M.charantia. The LC_{50} Values for adult snails using aqueous, methanolic and ethanolic extract of *V.amygdalina* were (537.3, 388.46 and 296.26ppm) and (473.49ppm, 388.46ppm and479.84ppm) for *M.charantia*. The corresponding LC_{90} values for adult snails using *V.amygdalina* were (762.8ppm, 450.45ppm and 597.56ppm) and (737.22ppm, 450.45ppm and 619.18ppm) for *M.charantia*. There were strong correlation between mortalities observed and concentration of both extracts. The result obtained shows that *V.amygdalina* and *M.charantia* are promising plants molluscicides and the molluscicidal components deserve further studies.

***Keywords*— Bulinus Globosus, Lethal Concentration, Momordica Charantia and Vernonia Amygdalina**

I. INTRODUCTION

The transmission cycle of *Schistosoma* species require specific freshwater snails as intermediate hosts [1], [2]. It is generally considered that snail control is one of the most rapid and effective means of reducing transmission of Schistosomiasis [3] - [6]. At present, the most reliable method of achieving drastic reductions in snail population density in short term is through the use of molluscicide [4]. Many different chemical molluscicides have been used in the past, but at present only one molluscicide, bayluscide is used [4], [7]. However, the high cost of Bayluscide, its impact on the community and its environmental effects have stimulated interest in search for alternative molluscicides of plant origin, in the control of human Schistosomiasis.

II. REVIEW OF LITERATURE

The use of indigenous plants rather than imported materials in the control of schistosomiasis is a welcome development, mostly as control programmes should be based on long term operation thereby saving cost. The search for alternative Molluscicides are still ongoing. Today, molluscicide is regarded as an important aggressive strategy in the control of the snail hosts of these diseases [8], [9]. In Nigeria screening of local plants for molluscicidal activity is receiving attention some of these plants that have been worked on include *Cymbopogan citratus* [10] *Carica papaya*, *Terminalia catappa* [11], *Securidaca longepedunculata*, *Tephrosia bracteolata* [12], *Hyptis suaveolens* [13]. Plants with molluscicidal activity may be exploited to contribute to schistosomiasis control especially if they are already grown locally for other purposes.

Vernonia amygdalina is shrub almost reaching the size of a tree up to 5m high. The plant occurs in gardens and it is a popular medicinal plant in Nigeria, used in treating bleeding, diabetes, yellow fever and many other diseases including microbial infections. It is anthelmintic. It has analgesic properties. It is used by farmers to check diseases in their crops [14].

Momordica charantia, is a climber, with yellow flowers, fruit ripening orange, 4-6cm long, bursting in 3 valves to expose the carmine red seeds; common in the tropics especially in moist regions. The plant is a popular medicinal plant in Nigeria used by the Yoruba, Igbo and Hausa, and the other tribes like Urhobo and others for convulsion, nervous disorder, gastroenteritis, including cholera; diabetes as anthelmintic crude drug, as purgative, as galactogogue, as liniment for leg, and for eyes problem. Skin problems, sexual problems, hormonal problems in women infertility, anaemia [14]. However, no work has been reported so far on the molluscicidal activity of *V.amygdalina* and *M.charantia* in Nigeria.

This study is aimed at evaluating the aqueous, methanolic and ethanolic leave extracts of *V.amygdalina* and *M.charantia* for molluscicidal activities.

III. MATERIALS AND METHODS

A) Collection and preparation of plant materials

M. charantia was collected from secondary forest regrowth along Ikere-Ekiti road while *V.amygdalina* was collected from home garden within Ado Local Government in Ekiti State. The plants were taken to the Plant Science Laboratory at Ekiti State University where they were properly identified by the botanist at the laboratory with reference to the standard flora. Voucher specimens were deposited at the herbarium of Plant Science Laboratory at Ekiti State University.

The collected plants parts used were the leaves. The plants collected were air-dried at room temperature between 25^0C-30^0C for three weeks and from direct sunlight in order to prevent the ultra-violet rays of the sun from destroying the chemical content of the plants. The dried plant were then pulverized with the aid of the mortar and pestle into fine powdery form and stored until extraction for the experiment.

B) Collection of snails

Snails were collected from river Elemi in Ado Local Government of Ekiti State, Nigeria. The collection was done between 8:00am and 12:00noon in order to avoid coming in contact with the cercaria which are usually shed from infected snails during the day when temperature rises slightly above 28^0C. Scoop net made of wire mess size of 1-2mm in diameter was used to scoop the snail from the water, others seen attached to the aquatic plants were collected by collecting the aquatic plants into the net and shaking them off into the net with the net held in the water. Most of the snails attached to the plants loosened their hold on the plants and dropped into the net, then they were transferred to a container with vegetation from the river side. The collected snails were then transported to the Zoology laboratory of Ekiti State University for identification and onward studies. In the laboratory, the snails collected were identified using the snails' identification key [15] to properly note the species to work with. The snails used in the study were *Bulinus globosus*.

C) Maintenance of snails in the laboratory

The identified *Bulinus globosus* were maintained in ten different troughs of about 10litre-15litre capacity, with each trough containing at least ten (10) snails to 5litres of dechlorinated and well aerated water. Each trough contained well washed and sterilized sand which was placed at the bottom of the trough to support rooted aquatic vegetation. The plants help in two ways: they provide oxygen, and they afford a suitable surface on which beneficial algae accumulate and on which the snail can crawl and deposit their eggs. The Plastic films were used for collecting the eggs. The snails were fed with fresh *Lactuva sativa* (lettuce) and the aquaria was maintained by changing the water three times in a week or when necessary. They were allowed to lay eggs. The eggs were seen after about 7days of acclimatization to the laboratory. The polythene sheet which contains the egg masses was isolated by cutting the plastic area around the egg mass, they were then transferred to other container containing about 200ml of dechlorinated water, it was incubated [16] at room temperature between 26^0C - 28^0C and hatching to juveniles were seen after about7-9days.

D) Extraction of plant materials

Extraction of the powdered plant material was done using three solvents namely; aqueous, methanol and ethanol.

Extraction with Water:

The water extracts of each plant was prepared by soaking 10g of the pulverized powdered plant part in 500ml of water and stirred at interval for six hour, and then left for 24hrs to infuse properly. After then, it was filtered by using the whatman filter paper. Distilled water was used to adjust the filtrate to make it up to the 500ml mark. That served as the stock for the working solution which is taken as 100% (w/v) or 20000ppm. The stock was used to prepare a series of concentrations of 1%, 2%, 3%, and 4% respectively and then used for the experiment which was calculated as 200ppm, 400ppm, 600ppm and 800ppm.

Extraction with organic solvents:

Methanolic and ethanolic extracts of each of the pulverized powdered plant was prepared by soaking 10g of each of the plant powdered part in 500ml of methanol and ethanol respectively. It was left for 24 hour for proper infusion, after then it was filtered using the Whatman filter paper. The solvent was removed by using the Soxhlet extractor. To prepare the stock solution, the methanolic and ethanolic extracts were adjusted to 500ml by the addition of distilled water. Series of concentrations of 1%, 2%, 3%, and 4% were prepared from the stock solution, which served as 200ppm, 400ppm, 600ppm, and 800ppm respectively.

E) Molluscicidal tests of aqueous plant extracts on adult and juvenile B. globosus

Adult and juvenile *B.globosus* snails of similar sizes were selected from the laboratory bred snail. The immersion method [17] was used. Different volumes of the aqueous extracts of the plants were measured out as 5ml, 10ml, 15ml, and 20ml from the stock solution and added to an equal volume of distilled water in 250ml beaker to have a working solution. For each concentration of the plant extract, group of 10 adult and 10 juvenile *B.globosus* were used with 5 adult and 5 juvenile snails per beaker each. For each experimental concentration, duplicates were prepared. Control experiments were performed in distilled water to run in parallel with the experiment. In all test 24hrs exposure period was applied. The snails were prevented from crawling out of the beaker by tying a fine mess over it with a rubber band. The snails were not fed during the course of the experiment. It had been observed that healthy snails live up to five days or more without food, provided other environmental condition are constant [11]. After the exposure period, the snails were washed thoroughly in tap water and were allowed a recovery period of 24hrs. Snails were considered dead by lack of movement by tactile stimulation of the operculum and were either retracted well into the shell or hanging out of the shell. Mortality count was taken after 24 hrs of recovery period.

F) Molluscicidal tests of methanolic and ethanolic plant extracts on adult and juvenile B. globosus

The immersion method [17] was used. Different volumes of the methanolic and ethanolic extracts of the various plants in 5ml, 10ml, 15ml, and 20ml from the stock solution were added to an equal volume of distilled water in 250ml beaker to have a working solution. The concentration for each solution was calculated as 200ppm, 400ppm, 600ppm, and 800ppm. For each concentration of the different plant extract, group of 10 adult and 10 juvenile *B. globosus* were used with 5 adult and 5 juvenile snails per beaker each. For each experimental concentration, duplicates were prepared. Control experiments were performed in distilled water to run in parallel with the experiment. In all test 24hrs exposure period was applied. After the exposure period, the snails were washed thoroughly in tap water and were allowed a recovery period of 24hrs. Mortality count was taken after 24 hrs of recovery period.

Statistical analysis:

The data obtained from the experiment were subjected to probit analysis software, Biostat 2009, Pro 5.9.8 professional package to obtain the LC_{50} and LC_{90}, Regression line equation, R square values and chi-square at 5% level of significance.

IV. RESULTS

Toxicity of aqueous extracts of V.amygdalina and M.charantia on Juvenile and Adult B.globosus

Molluscicidal activities of the water extracts of *V.amygdalina* and *M.charantia* from Table I shows the LC_{50} and LC_{90} values, which are the extracts concentration required to kill 50% and 90% of the juvenile and adult *B. globosus*, *V.amygdalina* aqueous had LC_{50} for juvenile and adult snails as 534.05ppm and 537.3ppm while the LC_{90} were 729.83ppm and 762.8ppm respectively. *M. charantia* had LC_{50} value for juvenile and adult snails as 558.99ppm and 473.49ppm and LC_{90} values as 622.63ppm and 737.22ppm respectively for the juvenile and adult snails.

The percentage mortality from fig. 1and 2 showed that at 200ppm concentration of the aqueous extracts of both plants, there were no records of death ,but when the juvenile and adult snails were subjected to the highest concentration of the extracts of 800ppm, there were no survivors from either plants extracts. The regression equation of the toxicity of *V.amygdalina* and *M.charantia* aqueous extracts on the juvenile and adult *B.globosus* as obtained from Probit analysis is shown in TableI. *V.amygdalina* aqueous extract was potent against the juvenile and adult *B.globosus*{juvenile at 24 hrs(x^2=1.64,df=3;p<0.05), and adult at 24hrs(x^2=2.1,df=3;p<0.05)}. The aqueous extract of *V.amygdalina* leaves showed that R^2=0.8824 and 0.9143 on the juvenile and adult *B.globosus* respectively. There were positive correlations between mortalities observed in the juvenile and adult *B.globosus* and the aqueous extracts concentrations of *V.amygdalina* leaves. *M. charantia* leaves aqueous extract was also potent against the juvenile and adult *B.globosus*{juvenile at 24hrs(x^2=3.86,df=3;p<0.05) and adult at 24 hrs(x^2=1.25,df=3;p<0.05)}. *M.charantia* aqueous extract showed that juvenile and adult *B.globosus* had R^2=0.8699 and 0.9846 respectively. There were strong positive correlation between mortalities observed in juvenile and adult *B.globosus* and aqueous extract of *M.charantia*.

Toxicity of methanolic extracts of V.amygdalina and M.charantia on Juvenile and Adult B.globosus

The results of the methanolic extracts of *V.amygdalina* and *M.charantia* against the juvenile and adult *B.globosus* are indicated in Table II. The LC_{50} and LC_{90} values of methanolic extracts reveals that the methanolic extract was more potent compared to the aqueous extract. The lethal concentration of *V. amygdalina* methanolic extract that killed 50%(LC_{50}) of juvenile and adult *B.globosus* were 182.86ppm and 388.46ppm, and the LC_{90} were 414.89ppm and 450.45ppm respectively. *M.charantia* methanolic leave extract had LC_{50} against juvenile and adult *B.globosus* as 269.84ppm and 388.46ppm and LC_{90} as 419.73ppm and 450.45ppm respectively. The percentage mortality from fig. 4 and 5 for the juveniles and adult *B.globosus* shows that **t**he regression equation of the toxicity of the methanolic extracts of these plants on the juvenile and adult *B.globosus* is shown in table II, *V.amygdalina* methanolic extract was very potent against juvenile and adult *B.globosus*{juvenile at 24hrs (x^2= 1.56,*df*=3;p<0.05). *V.amygdalina* methanolic extract concentration had R^2=0.8909 and 0.8629 on the juvenile and adult snail respectively. There were strong positive correlations between mortalities observed in the snails and *V.amygdalina* methanolic extract concentration. *M.charantia* methanolic extract had R^2=0.7529 and 0.8627 on juvenile and adult snail respectively. *M.charantia* methanolic leave extract concentration was potent against juvenile and adult *B.globosus* {juvenile at 24hrs(x^2=2.12,*df*=3;p<0.05) and adult at 24hrs(x^2=1.6,*df*=3;p<0.05)}. *M.charantia* methanolic extract had R^2=0.7529 and 0.8627 on juvenile and adult snail respectively. There was strong correlation between mortalities observed in the juvenile and adult *B.globosus* and methanolic extract of *M.charantia.*

Toxicity of ethanolic extracts of V.amygdalina and M.charantia on Juvenile and Adult B. globosus

The data in table III shows that the ethanolic extracts of *V.amygdalina* and *M.charantia* were potent on the snails. The LC_{50} and LC_{90} values obtained were as follows: *V. amygdalina* had LC_{50} against juvenile and adult *B.globosus* as 208.81ppm and 296.24ppm, and LC_{90} as 424.21ppm and 597.56 ppm respectively. *M.charantia* ethanolic extract had LC_{50} against juvenile and adult *B.globosus* as 236.9ppm and 479.84ppm and LC_{90} as 1029.48ppm and 619.18ppm respectively.

V. amygdalina ethanolic extract leave extract was the most potent ethanolic extract against juvenile *B.globosus* {juvenile at 24hrs(x^2=1.56, *df*=3; p<0.05)}. Adult at 24hrs showed (x^2= 0.14, *df*=3; p<0.05). The ethanolic extract of *V.amygdalina* showed that R^2=0.8909 and 0.9657 on the juvenile and adult *B.globosus* respectively. There was positive correlation between mortalities observed in juvenile

and adult *B.globosus* and the ethanolic extract of *V.amygdalina*.

Momordica charantia ethanolic extract was potent against juvenile and adult *B.globosus* {juvenile at 24hrs(x^2=2.54, *df*=3; p<0.05) and adult at 24hrs (x^2=3.86, *df*=3; p<0.05)}.

M.charantia ethanolic extract had R^2=0.5158 and 0.8699 on juvenile and adult *B.globosus* respectively. There was strong positive correlation between mortalities observed in the juvenile and adult *B.globosus* and *M.charantia* ethanolic leave extract.

TABLE I: TOXICITY OF AQUEOUS EXTRACTS OF *V.AMYGDALINA* AND *M.CHARANTIA* ON JUVENILE AND ADULT *B. GLOBOSUS*

Plants species	Snail stage	Regression equation	Chi square(p<0.05)	LC50(ppm)*	LC90(ppm)*
Vernonia amygdalina	Juvenile	y=-1.5+0.0075x	1.64	534.05	729.82
	Adult	y=-2.0+0.008x	2.1	537.3	762.8
Momordica charantia	Juvenile	y=-2.5+0.0095x	3.86	558.99	622.63
	Adult	y=-1.5+0.0080x	1.25	473.49	737.22

**mean lethal concentration*

TABLE II: TOXICITY OF METHANOLIC EXTRACTS OF *V.AMYGDALINA* AND *M.CHARANTIA* ON JUVENILE AND ADULT *B. GLOBOSUS*

Plants species	Snail stage	Regression equation	Chi square(p<0.05)	LC50(ppm)*	LC90(ppm)*
Vernonia amygdalina	Juvenile	y=2.5+0.0035x	1.56	182.86	414.89
	Adult	y=-1.0+0.0085x	1.68	388.46	450.45
Momordica charantia	Juvenile	y=-0.5+0.0080x	2.12	269.86	419.73
	Adult	y=-1.0+0.0085x	1.68	388.46	450.45

TABLE III: TOXICITY OF ETHANOLIC EXTRACTS OF *V.AMYGDALINA* AND *M.CHARANTIA* ON JUVENILE AND ADULT *B. GLOBOSUS*

Plants species	Snail stage	Regression equation	Chi square(p<0.05)	LC50(ppm)*	LC90(ppm)*
Vernonia amygdalina	Juvenile	y=2.5+0.0035	1.56	208.81	424.27
	Adult	y=0.0+0.0065x	0.14	296.24	597.56
Momordica charantia	Juvenile	y=1.5+0.0035x	2.54	236.9	1029.48
	Adult	y=-2.5+0.0095x	3.86	479.84	619.18

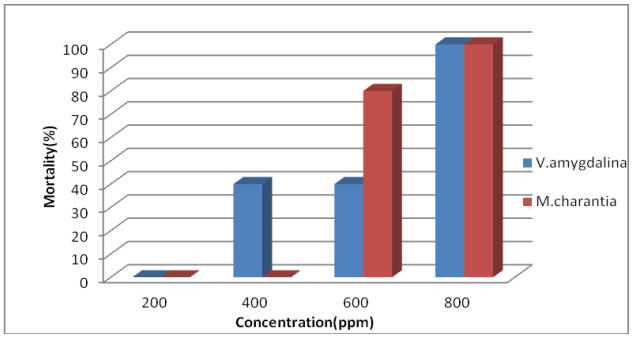

Fig. 1:*V.amygdalina* and *M..charantia* aqueous extracts against juvenile *B.globosus*

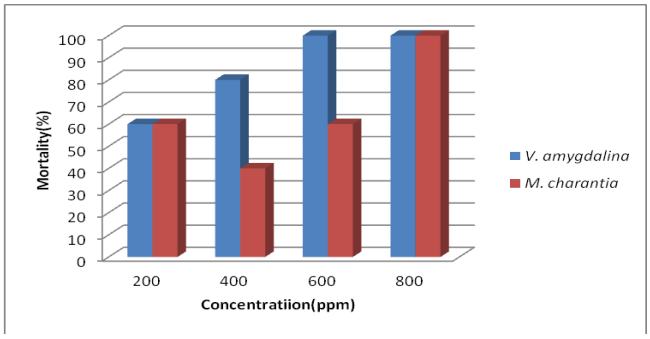

Fig.5: V.amygdalina and M.charantia ethanolic extracts against juvenile B.globosus

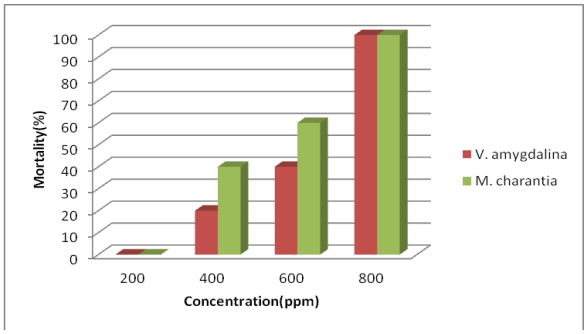

Fig. 2: *V.amygdalina* and *M.charantia* aqueous extracts against adult *B.globosus*

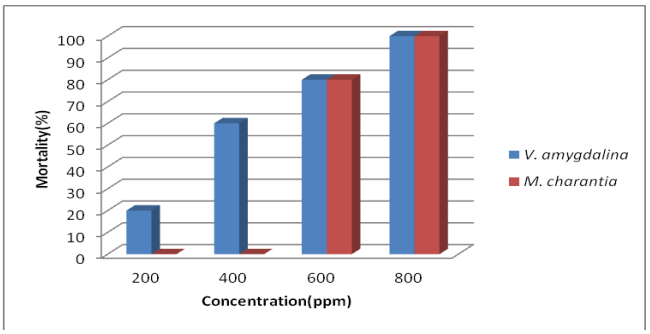

Fig. 6: *V.amygdalina* and *M.charantia* ethanolic extracts against adult *B.globosus*

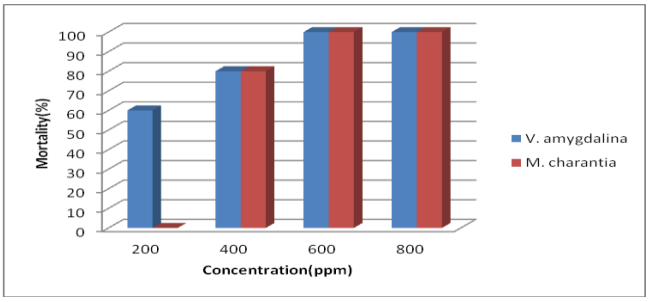

Fig. 3: *V.amygdalina* and *M.charantia* methanolic extracts against juvenile *B.globosus*

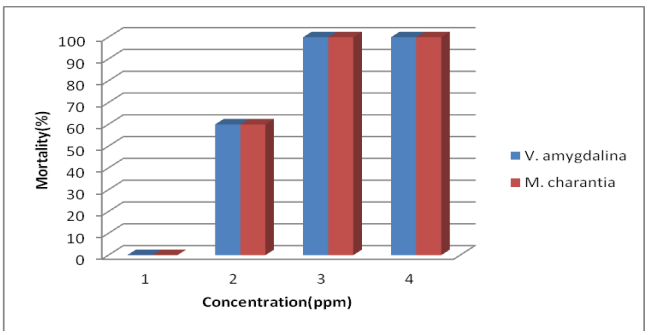

Fig. 4: *V.amygdalina* and *M.charantia* methanolic extracts against adult *B.globosus*

V. DISCUSSION

Molluscicidal activity of Vernonia amygdalina

In the present study, *V.amygdalina* methanolic leave extract that killed 50%(LC_{50}) and 90%(LC_{90}) of juveniles were 182.86ppm and 414.89ppm respectively, and for the adults, the lethal concentration that killed 50%(LC_{50}) and 90%(LC_{90}) were 388.46ppm and 450.45ppm respectively (Table II). The potency of *V.amygdalina* methanolic extract on the adult *B.globosus* was much higher when compared to the aqueous and ethanolic extracts of the same plant used in the present study. The aqueous extract of *V.amygdalina* in the present study on juvenile snail had LC_{50} and LC_{90} as 534.05ppm and 729.82ppm, and the LC_{50} and LC_{90} against the adult snails were 537.05ppm and 762.80ppm respectively. However the ethanolic extract of *V.amygdalina* was also potent on the juvenile and adult *B.globosus* but still less potent than the methanolic extract of the same plant with LC_{50} and LC_{90} on the juvenile snails as 208.81ppm and 424.27ppm and also LC_{50} and LC_{90} against the adult snails as 296.24ppm and 597.56ppm respectively. From the present study, methanolic extract of *V.amygdalina* was more toxic than the water and ethanolic extract on the snails. This is in line with [18] who revealed that the methanolic extracts of *Nicotiana tabacum*, *Afromomun citratum*, *A.meleguela*, *Piper guinense* and *Ocimum basilicum* were more toxic to *Bulinus truncates* and *Bulinus camerunensis* than the water extracts of the same plants.

Molluscicidal activity of Momordica charantia

Aqueous, methanolic and ethanolic extracts of *M.charantia* against juvenile and adult *B. globosus* were all moderately potent in the present study. The methanolic extract of *M.charantia* had LC_{50} and LC_{90} on the juvenile snails as 269.86ppm and 419.73ppm, and also LC_{50} and LC_{90} as 388.46ppm and 450ppm on the adult snails respectively. This activity was higher than the aqueous extract of the same plant in the present study. However, the ethanolic extract of *M.charantia* that killed 50%(LC_{50}) of the juvenile was the most potent of all the three extracts of *M.charantia* with LC_{50} as 236.9ppm. Due to the toxic nature of the ethanolic extract on the juvenile snails also, the snails were seen crawling out of the extract as the concentration increased hence the decline in mortality in the juvenile snails which had LC_{90} as 1029.48ppm (Table III). The continuous crawling out of the snails from the extracts solutions exposed to the sub-lethal concentration of the test extract is taken as an irritative, avoidance behavior similar to that described by [19], [20] in the pulmonate snails, *Bulinus.* The effect of *M.charantia* ethanolic extract on adult snail was moderately potent with LC_{50} and LC_{90} as 479.84ppm and 619.18ppm. In the present study based on LC_{50} and LC_{90} values, *M. charantia* demonstrated less molluscicidal activity than *V. amygdalina* this can probably be attributed to the difference in each plant active ingredient their mode of action and method of penetration of the snails.

VI. CONCLUSION

From the present study, *Vernonia amygdalina and Momordica charantia* could be considered as promising molluscicide in the control of snails intermediate host of Schistosomiasis.

VII. RECOMMENDATION

Phytochemical investigation to really identify the bioactive ingredients responsible for the molluscicidal potency is recommended. Research in the control of this intermediate host of Schistosomiasis using these natural plants product should be encouraged by the government because plants molluscicides could be one of the best means for the control of these snails intermediate host

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REFERENCES

[1]. Engels D, Chitsulo L, Montresor A, Savioli L. (2002). The global epidemiological situation of schistosomiasis and new approach to control and research. Acta Tropica; 82: 139-146.

[2]. Zhou XNM, Yang GJ, Yank K, Wang XH, Hong QB. Potential impact of climate change on schistosomiasis transmission in China. Am. J. Trop Med Hyg 2008; 78:188-194.

[3]. Klump H, Chu KY. (1980) Importance of aquatic weed *Ceratophyllum* to transmission of *Schistosoma haematobium* in Volta Lake, Ghana. Bulletin of the World Health Organization; 56:791-798.

[4]. McCullough FS, Gayral PH, Duncan J, Christie JD. (1980) Molluscicides in Schistosomiasis control. Bulletin of the World Health Organization; 58: 681-689.

[5]. Fenwick A, Cheesmond AK, Kardaman M, Amin MA Manjing BK. (1982)Schistosomiasis among the labouring communities in the Gezira irrigated area, Sudan. Journal of Tropical Medicine and Hygiene; 88: 3-11.

[6]. Manal A. (2010) Strategic control of schistosome intermediate host. Asian Journal of Epidemiology, 3(3):123-140.

[7]. Osman EA. (2000) Studies on transmission and control of Schistosomiasis in irrigation canals in Khartoum State. Ph. D thesis, Department of Zoology. Faculty of Science, University of Khartoum.

[8]. Giovanelli A., Silva C.L., Medeiros L. and Vasconcellos M.C. (2001): The molluscicidal activity of the latex of *Euphorbia splendens* var. *hisloppii* on *Melanoides tuberculata* (Thiaridae), a snail associated with habitats of *Biomphalaria glabrata* (Planorbidae). Mem. Inst. Oswaldo Cruz, 96:123-125.

[9]. Mello-Silva C. C., Vasconcellos M. C., Pinheiro, J. and Rodrigus M. L.A. (2006): Physiological changes in *Biomphalaria glabrata* (Say), (Pulmonata: Planorbidae) caused by sublethal concentrations of the latex of *Euphorbia splendens var. hisloppii* N.E.B. (Euphorbiaceae). Mem Inst Oswaldo Cruz. 101 (1): 38.

[10]. Benson Otarigho and Olajumoke A. Morenikeji (2012), Molluscicidal Effects of Aqueous and Ethanolic Extracts of Lemongrass (*Cymbopogon citratus*) Leaf against the Different Developmental Stages of *Biomphalaria Pfeifferi NY Sci J* 2012;5(8):70-77]. (ISSN: 1554-0200).

[11]. Adetunji V.O, Salawu O.T. (2010), Efficacy of methanolic leaf extracts of Carica papaya and Terminalia catappa as molluscicides against the snail intermediate hosts of schistosomiasis. J.med.plant.Res., 4(22):2348-2352.

[12]. Olofintoye L.K. (2010) Comparative evaluation of molluscicidal effect of *Securidaca longepedunculata* (Fres) and *Tephrosia bracteolata* (Guillano Perr) on *Bulinus globosus.* Journal of parasitology and vector Biology, Vol. 2(3), pp: 44-47.

[13]. Salawu, O.T and Odaibo, A.B. (2011). The molluscicidal effect of Hyptis suaveolens on different stages of *Bulinus globosus* in the laboratory. African journal of Biotechnology, Vol. 10(50), pp: 10241-10247.

[14]. Gill L. S. (1992): Ethnomedical Uses of Plants in Nigeria Uniben Press, University Benin, Benin City, pp: 1-450.

[15]. WHO. Meeting of directors of collaborating laboratories on molluscicide testing and evaluation. Document No. WHO/ SCHISTO/ 17-6, Geneva 1971.

[16]. Madsen H. (1987). Effects of Calcium concentrate on the growth and egg laying of *Helisoma duryi, Biomphalaria alexandrina, B.camarunensis and Bulinustruncates*(Gastropoda:planorbidae), J. Appl. Ecol, Vol. 24, pp: 823-836.

[17]. Komiya, Y., Y. Hosaka and K. Yasuraoka, (1962). Study for the Standardization of quantitative test of the susceptibility of Oncomelania snails to Sodium pentachlorophenate (NaPcP). Jpn. J. Med. Sci. Biol., 15(1): 41-51.

[18]. Ndamukong K.J.N, Ntonifor N.N, Mbu H.J, Atemn Keng A.E. and Akam M.T (2006) Molluscicidal activity of some Cameroonian plants on Bulinus species. East Afr.Med. J. 83:(3), 2006.

[19]. Evans NA, Whitfield PJ, Squire BJ, Fellows LE, Evans SV, Millott SM. Molluscicidal activity in the seeds of *Millettia thonningii* (leguminosae: papillionioidae). Trans. R. Soc. Trop. Med. Hyg., 1986, 80: 451-453.

[20]. Brackenbury TD. Gross histopathological effects of an extract of Agave attenuate on the epithelium of the digestive tract of *Bulinus africanus*. Ann. Trop. Med. Parasitol 1999, 93(5): 519-526.