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Research Article

Molluscicidal Efficacy of *Vitellaria paradoxa* (Shea Butter) Leaf extracts on *Schistosoma haematobium* Snail Host, *Bulinus globosus*

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ABSTRACT
Schistosomiasis is considered as one of the most important trematode diseases of man. This research was conducted to determine the phytochemical components and molluscicidal efficacy of methanol and aqueous extracts of *Vitellaria paradoxa.* Molluscicidal efficacy of the plant was tested on *Bulinus globosus*, the *Schistosoma haematobium host* snail species. The molluscicidal activity was assessed by determining the efficacy of various graded concentrations (0, 16, 32, 64 and 128mg/L) of the aqueous and methanol extracts. The crude extract collected from the plant was screened for phytochemicals and the result revealed the presence of Alkaloids, Tannins, Saponins, Oxalate, Flavonoid, Steroid, Terpene, Phlobotannin, Cardiac glycoside and Anthraquinone illustrating that there are bioactive components in the plant. The molluscicidal test showed that the mortality was dose – dependent. The highest molluscicidal activities were recorded at 64mg/L at 4hours and 128mg/L at 1hour of exposure to the methanol extract as well as 64mg/L at 24hours and 128mg/L at 12hours of exposure to the aqueous extract. The LC_{50} and LC_{90} of the methanol extract at 24hours were 20.74 and 53.92mg/L and at 48hours were 0.00 and 49.65 while the LC_{50} and LC_{90} of the aqueous extract at 24hours were 22.22 and 3.11mg/L and at 48hours were 1.81 and 2.95mg/L respectively. The result obtained showed that both extracts had significant ($p<0.05$) effect on *Bulinus globosus* with the methanol extract showing the highest potency at shortest time. Thus, the plant *Vitellaria paradoxa* can be of great help in the management of the snail host of Urinary schistosomiasis.

Keywords: Anthraquinone; Oxalate; Phlobotannin; Phytochemicals; Tannins; Terpene; Schistosomiasis

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INTRODUCTION

Schistosomiasis, also known as Bilharzia (after a German physician, Theodor Bilharz, who first discovered and described these worms working in Egypt in 1851) is a fresh water borne disease caused by parasitic trematodes of the Genus *Schistosoma* (Family; Order; Class; Phylum; Kingdom) which are the only digenic trematodes to inhabit homoeothermic vertebrates (Webster, 2006). Schistosomiasis is of international concern and categorized as a Neglected Tropical Disease due to its high level of prevalence all over the world, high cost of treatment as well as control and effect on socio-economic and human development in developing countries (Vennervald and Dunne, 2004; WHO 2016) giving it a place as the most parasitic disease after malaria among the infectious diseases of tropical and subtropical countries and the 3rd most prevalent parasitic disease in the world (Gehad *et al*., 2009) infecting more than 237 million people with 700 million at risk in 77 countries worldwide, Africa accounting for 80-85% of infection (WHO, 2015) of which Nigeria is the most affected country in the World with prevalence estimated at 101.28million people at risk of infection while 25.83 million are infected including a large percentage of school children. The rife of infection is ascribed to poor environmental sanitation, inadequate access to safe water, low socio-economy, favourable climate for transmission of the disease, construction of dams, expansion of irrigation based agricultural schemes

and population movements, human behavioural and cultural practices leading to seemingly continuous exposure of the trematode to humans (Woldmichael *et al*., 2013). The mortality of schistosomiasis is worrisomely high as a result of expensive and/or high cost of treatment, late diagnosis and little or no facilities to detect disease in the rural areas where the disease is most prominent.

Schistosomiasis over time has posed a serious threat with the upsurge in mortality on children especially in the Tropical and sub-tropical regions of the world, thus there is need to find a cheap, available and long-lasting method of control. Various control methods have been used of which includes; Reduction of human contact with water, improved sanitation, Reduction of worm burden or egg production by the parasite through chemotherapy, Modification of the ecology of the snail habitat etc. These methods have yielded little or no results due to poor socio-economic conditions of the population and high cost of control. Therefore, the best approach remains, the eradication or reduction of snail population which has been widely accepted as a workable solution to the problem.

However, it is important to note that a very large proportion of the population are unaware or have little knowledge of the disease especially in the rural areas, thus, posing difficulties in diagnosis and proper control of this disease. Furthermore, it is expedient that detailed information on infection rates, population studies and snail survey be carried out especially in Nigeria to ascertain the actual rate of infection for the elimination of the menace, schistosomiasis.

Due to the increasingly worrisome rate of morbidity and mortality; Schistosomiasis is classified by the World Health Organisation (WHO) as a Neglected Tropical Disease (NTD). Specifically, there are two (2) forms of Schistosomiasis; Urinary and Intestinal schistosomiasis, both carried by intermediate snail species; hence, there is a need to break the parasitic link or cycle by eliminating the carrier snail host. In respect to this, over time, molluscicides have been used to control snail but due to their proven hazardous effects to the environment and high cost, the use of biological means is called for of which medicinal plants in this case have paved way for research to not just evaluating their mollusicidal efficacy but for remedy to many diseases. Therefore, this work is aimed at evaluating the plant, *Vitellaria paradoxa* for its efficacy on intermediate snail host of Urinary Schistosomiasis; *Bulinus globosis* as safer, cheaper, and readily available and more efficient modality to curb the disease Schistosomiasis worldwide and encourage natural evaluations on cures for various diseases to reduce the adverse effects of synthetic products.

MATERIALS AND METHODS

Sample Collection

Test snails

The adult freshwater snails were collected from a stagnant pool of water at Central Mosque, Federal University of Technology, Minna, Niger State by handpicking. The snails were thereafter for taken to the Department of Animal Biology, Federal University of Technology, Minna for identification. The snail species was thus identified as *Bulinus globosus* and arranged separately in plastic disposable plates with capacity of about 500ml of distilled water with stocking density of 10 snails per plate under laboratory condition for acclimatization which lasted for 2 weeks in the Department of Animal Biology, Federal University of Technology, Minna at a temperature of 28^0C - 31^0C. Slightly boiled and cooled lettuces (*Lactuca sativa*) were used to feed the snails at 2-day interval prior to the commencement of the experiment.

Plant materials

The plant species, *Vitellaria paradoxa* leaves were collected by handpicking at Maryam Babangida Secondary school, Bosso Local Government Area, Minna, Niger state and taken to the Herbarium of the Department of Biological sciences, Federal University of Technology, Minna for identification and authentification. The plant leaves were thereafter rinsed thoroughly with distilled water to remove dust, sand and unwanted materials. 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 leaves were later on blended into powder and homogenized to a fine powder using a sieve and stored in 2 air tight bottles for aqueous and methanol extracts respectively with each bottle containing 50g of powdered leaf sample.

Ethanol extraction

50 g of the powdered leaf was soaked in 500 ml of 100% methanol for two days (48h) with vigorous shaking at 4hour intervals. The solutions were filtered using filter paper (Whatman No. 1) and subjected to evaporation using laboratory water bath located in the Department of Biological sciences, University of Technology, Bosso, Minna, Niger State. The evaporation process took 3 days leaving slurry materials which constituted the crude ethanol extract (Das *et al*., 2010).

Aqueous extraction

100 g of the powdered leaf sample was soaked in 800 ml of distilled water for two days (48h),

vigorously shaken at 4-hour intervals to extract polar materials. The solutions were filtered using filter paper (Whatman No. 1) and allowed to evaporate using the water bath located at the Department of Biological Sciences, Federal University of Technology, Minna, Niger State. The evaporation process took 7 days leaving materials which constituted the crude aqueous extract (Das *et al*., 2010).

Preparation of stock solution and serial dilution

The stock solution and serial dilution were carried out according to the standard method described by Otarigbo *et al*. (2013) and Adetunji *et al*. (2010). One gram of each crude extract was dissolved in 490ml of distilled water to prepare the stock solution (500ml). The different volumes of 0.00 (control), 16, 32, 64 and 128ml from the stock solution of the extracts (methanol and aqueous extracts respectively) of the plant were added to an equal volume 500ml of distilled water in plastic troughs to obtain the working solutions. Then the concentration of each solution was calculated in milligram Litre (mg/L).

Molluscicidal potency test

Ten (10) Adult snails of uniform size were immersed in 5X2 replicates. In each setup, the snails were prevented from crawling out by covering the troughs with fine mesh net and tied with rubber bands. The snails were not fed in course of the experiment. It has been observed that healthy snails can stay for five or more days without food provided other environmental conditions remain constant (Salawu *et al*., 2010). The snails were left in the various extract concentration setups for a total of 48hours with constant monitoring at time frames of 1, 4, 6, 12, 24 and 48 hours respectively. Dead snails were removed from the setups and recorded. Death of snails was determined by the lack of reaction to irritation of foot with a needle probe to elicit typical withdrawal method.

Phytochemical Analysis

Phytochemicals are non- nutritive plant chemicals that have protective or disease preventive properties. Presence of phytochemical components illustrate that there are bioactive components embedded in the plant. The phytochemicals which are present in the extracts of leaf of *V. paradoxa* were determined and quantified by standard procedures as described below (Hagerman *et al*., 2000; Obadoni and Ochuko, 2001; Kumaran and Karunakaran, 2006; AOAC, 2010).

Determination of Total Phenolic Compounds

The total phenolics content in solvent extracts was determined with the Folin- Ciocalteu's reagent (FCR). In the procedure, 100 mg of extract of the sample was weighed accurately and dissolved in 100mL of triple distilled water (TDW). One ml of this solution was transferred to a test tube, then 0.5 mL 2N of the Folin-Ciocalteu reagent and 1.5 mL 20% of Na2CO3 solution was added and ultimately the volume was made up to 8mL with TDW followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid.

Determination of Total Alkaloids

Five grams (5g) of the sample was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Determination of total tannins

Five hundred grams of the sample was weighed into a 50mL plastic bottle. Fifty milliliters of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50mL volumetric flask and made up to the mark. Then 5mL of the filterate was pipetted out into a test tube and mixed with 2mL of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Determination of Total Saponins

The samples were ground and 20 g of each were put into a conical flask and 100cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200mL 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250mL separatory funnel and 20mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Sixty milliliter of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated.

Total Flavonoid Determination

Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. One millimeter of test sample and 4mL of

water were added to a volumetric flask (10mL volume). Five minutes after adding 0.3mL of 5 % Sodium nitrite, 0.3mL of 10% Aluminum chloride was added. After 6 min incubation at room temperature, 2mL of 1M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10mL with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically (Shimadzu UV-1609, Japan). Results were expressed as catechin equivalents.

Test for Cardiac Glycosides

Five milliliter (5mL) of each extract was treated with 2 mL of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayed with 1mL concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

Test for Phlobatannins

Deposition of a red precipitate when 2mls of extract was boiled with 1mL of 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Test for Terpenoids

1ml of chloroform was added to 2mL of each extract followed by a few drops of concentrated sulphuric acid. A reddish-brown precipitate produced immediately indicated the presence of terpenoids.

Test for Quinones

A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or colouration).

Test for Oxalate

To 3mL portion of extracts were added a few drops of ethanoic acid glacial. A greenish black colouration indicates presence of oxalates.

Test for Steroids

Steroids were sought by the reaction of Liebermann. Ten (10mL) of ethanolic extract was evaporated. The residue was dissolved in 0.5mL of hot acetic anhydride; we added 0.5mL of the filtrate chloroform. Treated with the reagent of Libermann Burchardt, the appearance, at the interphase, a ring of blue-green, showed a positive reaction.

Data Analysis

Descriptive statistical analysis was performed using mean ± standard error of mean. The results relating to molluscicidal activities of the plant extract and tested concentrations were evaluated using Analysis of Variance and Duncan Multiple Range Test, with a significance of 0.05. The median and upper lethal concentrations were determined by regression analysis. All analyses were carried out using Statistical Packages for Social Sciences (SPSS) 20th version and Microsoft EXCEL 2010.

RESULTS

Phytochemical Assay of *Vitellaria paradoxa*

Phytochemical assay Qualitative phytochemical analysis of the extracts revealed the presence of all the tested active ingredients (tannin, saponin, flavonoids, alkaloids, oxalate, steroid, terpene, philoban, cardiac glycoside and anthraquinine), as shown in Table 1.

Methanol potency

The methanol extract showed a significant ($P<0.05$) mortality as the time of exposure and concentration increases except for 16 and 32mg/L in which there was no significant difference ($p>0.05$) in the mortality at 1hour of exposure. The highest mortality was recorded in 64mg/L at 4hours (10.00 ± 0.00) and 128mg/L at 1 hour (10.00 ± 0.00) with 100% mortality of all snails subjected to the above concentration. The medial (LC_{50}) and upper (LC_{90}) lethal concentration of the methanol extract was presented in Table 2. LC_{50} at 24 and 48hours were 20.74 and 53.92 while the LC_{90} at 24 and 48 hours were 0.00 and 49.65 respectively. The R^2 values recorded unchanged confirms the dependence of the mortality observed to the increment of plant extract after 24 hours. The LC_{50} values decreased from 20.74mg/L after 24 hours to 0.00 after 48 hours.

Aqueous potency

In the case of the aqueous extract, there was no significant difference in snail mortality at exposure periods of 1 hour to 12 hours in the 16, 32 and 64mg/L doses ($P>0.05$). A subsequent significant difference was observed at the increase of exposure period at 128mg/L ($P<0.05$). The extract showed highest significant snail mortality at exposure period of 24 hours at 64mg/L and 12 hours at 128mg/L (Table 3)

Aqueous potency

The LC_{50} at 24 and 48 hours were 2.22 and 1.81mg/L while the LC_{90} at 24 and 48 hours were 3.11 and 2.95 respectively. The decrease in LC_{50} and LC_{90} values and increase in R^2 value confirms the dependence of the mortality observed to the increment in plant extract. Both extracts had d highest snail mortality from the 64mg/L concentration with 100% mortality as well as in the 128mg/L concentration (Table 4).

Table 1: Bioactive phytochemicals of *Vitellaria paradoxa*

Phytochemicals constituents	Presence/Absence
Tannin	+ve
Saponin	+ve
Alkaloid	+ve
Steroids	+ve
Anthraquinone	+ve
Flavonoids	+ve
Oxalate	+ve
Terpene	+ve
Cardiac glycoside	+ve
Phlobatannin	+ve

Table 2: Mean molluscicidal effect of Methanol extract of *Vitellaria paradoxa* leaves on *Bulinus globosus* after 48hours exposure period

Sample	1hr	4hrs	6hrs	12hrs	24hrs	48hrs
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
16mg/L	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.50 ± 0.50^{a}	2.00 ± 0.00^{b}	4.00 ± 0.00^{b}	6.00 ± 0.00^{b}
32mg/L	1.00 ± 0.00^{a}	3.00 ± 0.00^{b}	3.00 ± 0.00^{b}	5.00 ± 0.00^{c}	7.00 ± 0.00^{c}	8.00 ± 0.00^{c}
64mg/L	6.50 ± 0.00^{b}	10.00 ± 0.00^{c}	10.00 ± 0.00^{c}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}
128mg/L	10.00 ± 0.00^{c}	10.00 ± 0.00^{c}	10.00 ± 0.00^{c}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}	10.00 ± 0.00^{d}

Values with the same superscript within a row are not significantly different at p>0.05

Table 3: Mean molluscicidal effect of Methanol extract of *Vitellaria paradoxa* leaves on *Bulinus globosus* after 48hours exposure period

Sample	1hr	4hrs	6hrs	12hrs	24hrs	48hrs
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
16mg/L	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.00 ± 0.00^{b}	3.00 ± 0.00^{b}
32mg/L	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.00 ± 0.00^{b}	4.50 ± 0.50^{c}
64mg/L	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	10.00 ± 0.00^{c}	10.00 ± 0.50^{d}
128mg/L	3.00 ± 1.00^{b}	6.50 ± 0.50^{b}	7.50 ± 0.50^{b}	10.00 ± 0.00^{b}	10.00 ± 0.00^{c}	10.10 ± 0.00^{c}

Values with the same superscript within a row are not significantly different at p>0.05

Table 4: Lethal concentration of Aqueous and Methanol extracts on *B. globosus*

Extract/Time (Hour)	LC_{50} (mg/L)	LC_{90} (mg/L)	R^2	Regression Equation
Aqueous extract				
24	2.22	3.11	0.75	Y=45x-50
48	1.81	2.95	0.85	Y=35x-13.333
Methanol				
24	20.74	53.92	0.96	Y=1.2054x+25
48	0.00	49.65	0.96	Y= 0.8036x+50

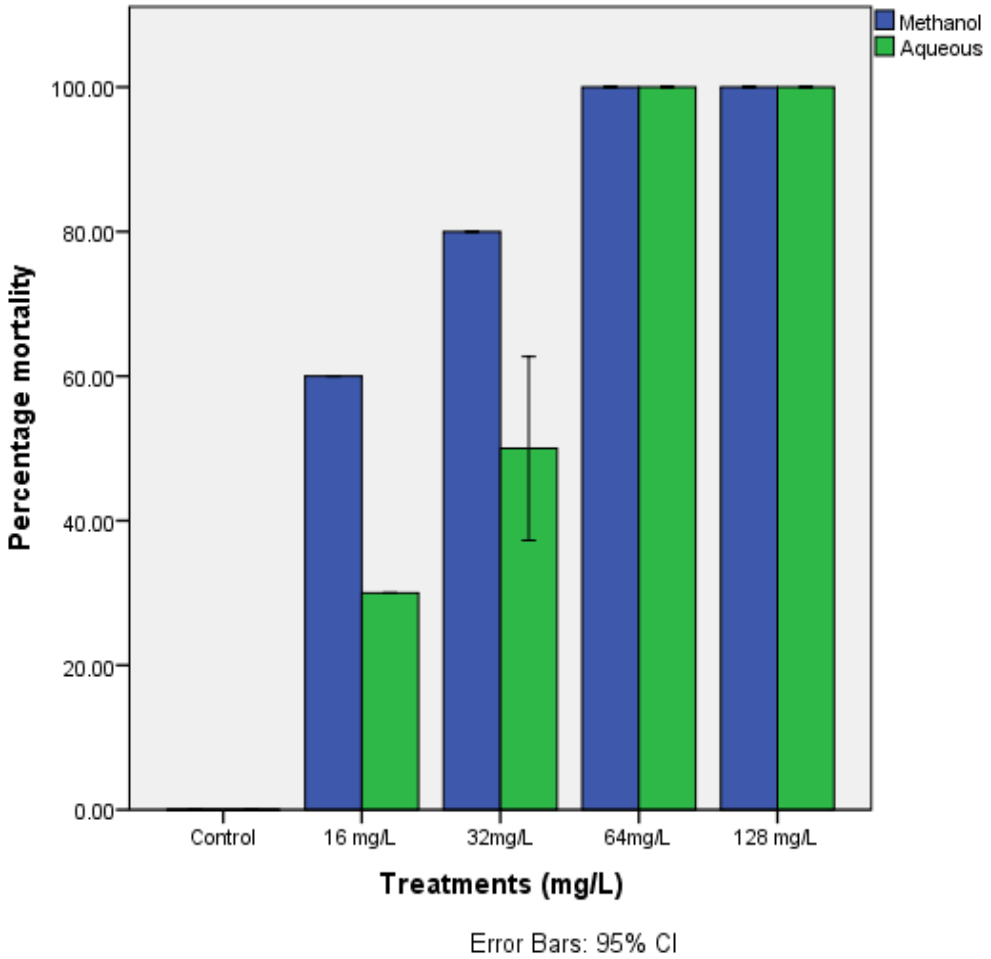

Figure 2: Percentage mortality of Mollusicidal efficacy of Aqueous and Methanol extracts on *B. globosus*

DISCUSSION

The environmental and economic problems attributed to the use of synthetic molluscicide stimulated the search for a safe, cheap, readily available and easily applicable alternative of plant origin. Therefore, most countries all over the world have paid great attention to the production, manufacturing and use of plant molluscicides. Thus, the present study is intended to search for ideal molluscicide of plant origin on the basis of dosage response for combating adult snail intermediate host of schistosomiasis.

The phytochemical analysis of the plant used in this study, *Vitellaria paradoxa*, showed the presence of some phytochemical compounds such as tannins, saponins, flavonoids, terpenes, steriods, oxalate, phlebotannin, anthraquinone and cardiac glycoside which may be responsible for the high molluscicidal potency of both extracts (methanol and aqueous). This finding agrees with the work of Singh (2013), who reported that in many plants, the activities of compounds on exposed animals was due to the presence of Saponin, terpenoid and alkaloid components of the plants. It is evident from the result section that the methanol and aqueous extracts used were toxic against *B. globosus* snails with the toxicity of the tested snails shown to increase with the increase in the concentration and time exposure. The observed sensitivity of adult towards higher concentration is constituent with Stednichenko (2010), Kirichuk *et al.* (2010) and Rawi *et al.* (2011) who reported that the uptake of plant compounds used could be time and concentration dependent leading to an increased effects on exposed animals. As reported by several investigators, elevated plant extract concentrations have been associated with an aggravated toxic response for several aquatic species. Although the methanol and Aqueous extracts had significant effect of mortality on the snail species, however in comparison, from the result section, it is clear that the methanol extract portrayed better effect at lower concentrations which may be due to high synergetic effect and polarity of methanol on the biochemicals and physiological function of the plant (Oskay and Sari, 2007). From the established regression lines, the molluscicidal activity based on LC_{50} values falls within the range of other plants that have been judged as promising molluscicides (Abdel-Hamis, 2003).

CONCLUSION

The result obtained from this study indicated that the use of *Vitellaria paradoxa* plant extracts was able to kill fresh water intermediate snail host *Bulinus globosus* of *Schistosoma haematobium* when used as an alternative option to synthetic molluscicides. Based on the findings of this research work, methanol can be considered as suitable solvent for the plant.

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