Original Research Article

Studies on the activity of aqueous extracts of selected medicinal plants against freshwater target and non target organisms

Abstract

The aqueous extracts of the stem bark and leaves of *Phyllanthus niruri* (a tropical small herb, commonly known as a Bhumi Amla), belonging to the family Phyllanthaceae and the aqueous extracts of stem bark and latex of *Euphorbia tirucalli* (a semi-arid tropical plant, commonly called milk bush) of the family Euphorbiaceae, were investigated as plant origin molluscicides, against freshwater snails, *Lymnaea* (Radix) *acuminata* (Lamark 1822) and *Indoplanorbis exustus* (Deshayes 1834), both snails are responsible for causing fascioliasis. A significant time and dose-dependent effect of aqueous extracts of both the plants against the freshwater snails were observed. Thus increase in exposure time, the LC_{50} of *Phyllanthus niruri* stem and leaf extracts were decreased from 267.89 mg DW/L (24h) > to 180.64mg DW/L (96h) against *Lymnaea acuminata* and 261.49 mg DW/L (24h)> to 136.40 mg DW/L (96h) against *Indoplanorbis exustus*. Likewise, the LC_{50} of aqueous extract of latex of *Euphorbia tirucalli* were decreased from 1.80 mg DW/L (24h) >to 0.65 mg DW/L (96h) against *Lymnaea acuminata* and 0.90 mg DW/L (24h)> to 0.28 mg DW/L (96h) against *Indoplanorbis exustus*, with the increase in exposure time. Its aqueous stem bark extract showed a similar pattern.

These aqueous plant extracts at higher doses were also observed to be lethal to freshwater fish *Channa punctatus*, which share a common habitat with the freshwater snails, but the doses LC_{90}, (24h) of snails didn't cause any mortality to these fishes, thus indicating that these plant extracts can be safely used as molluscicides.

Keywords: Molluscicides, Euphorbiaceae, *Lymnaea acuminata, Indoplanorbis exustus, Channa punctatus,* Phyllanthaceae

Introduction

The World Health Organization considers *Fascioliasis* and *Schistosomiasis* to be neglected zoonotic diseases. Recent research has validated fascioliasis inclusion on the list of major human parasitic illnesses. Fascioliasis is currently a vector-borne illness with the most extensive known latitudinal, longitudinal, and altitudinal distribution. Despite theoretical limitations connected to its biology, *Fasciola hepatica* has colonized five continents, despite being dependent on environmental and human activity. In hypo- to hyper endemic areas of Central and South America, Europe, Africa, and Asia, human fascioliasis displays a diversity of epidemiological characteristics that are linked to a wide range of environments. The presence of adequate aquatic snail intermediate hosts from the Lymnaeidae i.e. *Lymnaea acuminata* and Planorbidae *Indoplanorbis exustus* families is a significant aspect.

Over the last few decades, a variety of chemical molluscicides have been employed to treat Fascioliasis and other snail-borne infections. Copper sulphate and other copper salts are among the most notable, having previously served important roles but now being mostly abandoned due to low efficiency and inactivation by organic and inorganic materials in water. Despite the many advantages of utilizing synthetic molluscicides as mollusc control agents, they have the potential to disrupt entire ecosystems, inflicting harm to humans and non-target creatures. Due to their bioaccumulation and long-term persistence, synthetic pesticides have generated a high rate of toxicity in water body's organism. Plant molluscicides are receiving more attention from national and international agencies in the hopes of proving to be less expensive and more readily available than synthetic pesticides. Plant molluscicide research has become multidisciplinary as a

result plant products have been promoted in several countries due to their wide variety of desirable properties, including high toxicity, low cost, low mammalian toxicity, low water solubility, easy biodegradability, plentiful growth in endemic areas, and operator safety.

The present study reports the molluscicidal effects of aqueous extracts of different parts of two plants *Phyllanthus niruri* and *Euphorbia tirucalli* against harmful freshwater snails, *Lymnaea acuminate* and *Indoplanorbis exustus*, which serve as intermediate hosts for *Fasciola hepatica* and *Fasciola gigantica*, in causing fascioliasis. Toxicity tests were also carried out on freshwater fish *Channa punctatus* (which shares common habitat with the snails) to see if there was any environmental toxicity.

MATERIALS AND METHODS

Test Plants:

The plants under investigation were *Phyllanthus niruri* and *Euphorbia tirucalli*. Both the plants were collected easily, during the rainy season, from the Botanical Garden of D.D.U. Gorakhpur University, Gorakhpur. *Phyllanthus niruri* (commonly called bhumi amla), belongs to the family Phyllanthaceae, while *Euphorbia tirucalli* (commonly called milk bush or pencil tree) belongs to the family Euphorbiaceae, the milky latex of which is extremely irritating to the skin and is toxic and therefore should be carefully handled.

Preparation of aqueous extracts of Stem bark, and Latex

Stem bark: Fresh stem and leaves from *Phyllanthus niruri* and stem bark from *Euphorbia tirucalli* were minced with distilled water, homogenized for 5 minutes, and then centrifuged at 1000g for around 10 minutes. The molluscicidal activity of the obtained supernatant was tested**.**

Latex: By cutting the stem apex, the white latex from *Euphorbia tirucalli* was drained into glass tubes, and the latex was lyophilized at -40^{0}C, and the lyophilized powder was employed for

future use. To obtain the correct concentrations, the freeze-dried powder was combined with an appropriate volume of distilled water.

Test Animals:

The target organisms for this research study, adult freshwater snails, *Lymnaea acuminata* (2.5 ± 0.9cm in shell height), and *Indoplanorbis exustus*,(1.5 ± 0.2cm in shell height) were collected from pool alongside the campus of Veer Abdul Hameed P.G. College, Medical Road, Gorakhpur district. The collected animals were kept in glass aquaria containing de-chlorinated tap water to acclimatize to laboratory conditions. The water in the aquaria was changed every 24 hours. To prevent the water fouling any dead animal were removed periodically. As the non-target organism, freshwater fish, *Channa punctatus* (11.0±0.5 cm in total length), were captured from Ramgarh lake of Gorakhpur district.

Toxicity experiments:

The Singh and Agarwal (13) method was used to conduct the toxicity test for both snails, ten snails were housed in glass aquaria with 3L de-chlorinated tap water. The experimental snails were exposed for 96 h to four different concentrations of stem and leaves of *Phyllanthus niruri* and stem bark and latex of *Euphorbia tirucalli*

For each concentration, six similar aquaria were set up. The snails were grown in the same way as the control group, but without any toxicity treatment.

Toxicity of aqueous extracts of stem bark and leaves and latex of both the plants under research, were also studied in a mixed population of fish and snails as the fish share the habitat with the snails. Accordingly, 10 snails *Lymnaea acuminata* and 10 fish *Channa punctatus*, were placed together in 6L of de-chlorinated tap water, and these mixed populations of fish and snails were checked for toxicity for previously determined LC_{90} (24h) 0f snails for 24h.

Snail behavioral reactions were detected for up to 2 hours after the treatment. Every 24h, 48h, 72h, and 96h, mortality was recorded LC_{10}, LC_{50}, and LC_{90} values, upper and lower confidence limits (UCL LCL), slope value 't' ratio, 'g' factor and heterogeneity were determined using the POLO computer program (12) utilizing the probit log analysis approach regression coefficient was also determined between exposure time and different values of LC_{50} (14).

RESULT

Experimental conditions of water were calculated using APHA/WPCF method (1998)(1). Accordingly, the parameters and their values determined were as follows:

Atmospheric temperature	35.0 -36. 0^0C
Water temperature	28.0- 29.0^0C
pH of water	7.2-7.4
Dissolved Oxygen	6.9-7.4
Free carbon dioxide	4.6-6.7
Bicarbonate alkalinity	110.0- 111.0

(A) Effects on Behavioural changes and Poisoning Symptoms

Exposure to the aqueous extracts of root, latex, and stem bark of *Phyllanthus niruri and Euphorbia tirucalli*, caused significant behavioral changes in the freshwater snails *Lymnaea acuminata* and *Indoplanorbis exustus*, within 5-10 min of exposure to the toxicity test. Hyperactivity of the sluggish snails, during the first 30-40 min, was observed in the aquaria. With time, the snails got intoxicated and accordingly showed muscular twitching and spiral twisting, which resulted ultimately in paralysis, and finally into the death of the snails.

(B) Dose-mortality response

LC values ($LC_{10,50,90}$) of aqueous extracts of stem bark and leaves of *Phyllanthus niruri* and stem bark and leaves and latex of *Euphorbia tirucalli* for period ranging from 24h to 96h for the snails, *Lymnaea acuminata* and *Indoplanorbis exustus* have been given in (Tables 1-6). In case of both the snail toxicity was time as well as dose dependent. There was a significant

negative correlation between LC_{50} values and exposure time (Tables 1-6). Thus increase in exposure time the LC_{50} of *Phyllanthus niruri* aqueous stem bark and leaves extract , decreased from 267.89 mg DW/L (24h);> 225.13 mg DW/L (48h);> 205.10 mg DW/L(72h);> to 180.64 mg DW/L(96h) and 229.37 mg DW/L(24h);> 210.86 mg DW/L (48h)> 164.72 mg DW/L (72h);> to 136.40 mg DW/L (96h) in case of *Lymnaea acuminata* and *Indoplanorbis exustus*, respectively. Same trend of toxicity was observed in case of stem bark and latex of *Euphorbia tirucalli* against both the snails at all the exposure periods.

Laboratory experiments also indicate that the latex and stem bark extracts of both the plants were more toxic against *Indoplanorbis exustus* than *Lymnaea acuminata* at all the exposure periods.

At higher doses, active moiety of plants, which were effective against the snails, would also cause death amongst the fish. Consequently, a mixed population of 10 snails (*Lymnaea acuminate*) and 10 fish *(Channa punctatus*) were treated with the 24h, LC_{90} of stem bark of *Phyllanthus niruri* and latex and stem bark of *Euphorbia tirucalli*, up to the LC_{90} doses for snail *Lymnaea acuminata* there was no mortality amongst fish (Table 7).

The slope values given in toxicity tables (1-6) were steep and heterogeneity factor was less than 1.0 indicates the result found to be within the 95% confidence limits of LC values. The regression test ('t' ratio) was greater than 1.96 and the potency estimation test ('g' value) was less than 0.5 at all probability levels.

Discussion

Several plant species having specific active chemical constituents in them, have been identified as natural molluscicides, and they are now being investigated. Among recent studies,

some plant extracts have shown molluscicidal efficacy and saponins isolated from *Camellia oleifera* seeds have been used to create the novel chemical Luo-Wei, also known as tea-seed distilled saponin (TDS), which has been tested effectively against intermediate host snails *Oncomelania hupensis*, *Biomphalaria alexandrina*, and *Bulinus truncates* in China and Egypt (4).

Not until the mid-1960's was the first plant (*Phytolacca dodecandra*) used for control of schistosomiasis in an endemic focus, in Ethiopia (6). The discovery of *Phytolacca dodecandra* berries as a naturally available potent molluscicide in Ethiopia has raised much awareness of the use of plants as effective molluscicides, as they are effective in killing snail vectors while being safe to non-target organisms and easily degradable in the environment (7,3).

The molluscicidal properties of various leaf extracts prepared from tuba-tuba *Jatropha curcas* L. were tested against the freshwater snail (*Oncomelani*a *hupensi*s quadrasi), an intermediate host of *Schistosoma* sp., the causative agent of schistosomiasis, with the crude petroleum ether extract at a concentration of 1.5 mg/mL^{1} showing the highest mortality of 80% (8). The molluscicidal efficacy of aqueous extract of *Achyranthes aspera L*. (Amaranthaceae) on adult *Biomphalaria pfeifferi* and *Lymnaea natalensis* snails, which are of major medicinal and veterinary value in Ethiopia, has been recently established (9).

Among the bioactive plant compounds, the most prevalent terpene compounds are responsible for molluscicidal activity. Thymol and α-pinene both have been investigated to be active against *B. glabrata*, causing mortality in concentration dependent patterns and having a fatal effect at doses commensurate with WHO recommendations (LC_{90} of 7.11 and 10.34 g mL^{1}, respectively), and are also reported to be inhibitors of acetyl cholinesterase of *B. glabrata* snails (2). In a study, the molluscicidal action of *Moringa oleifera* flowers was tested in *Biomphalaria*

glabrata using an aqueous extract. In adult molluscs, this extract was fatal, with a lethal concentration of LC_{50}=2.37 mg/mL Also, fruits of the species *Randia nilotica* were employed on molluscs of the species *Biomphalaria pfeifferi* and *Bulinus truncatus* in the second aqueous extract. On both molluscs employed in the trials, these extracts showed molluscicidal action. The molluscicidal effect of aqueous extract of leaves from the species *Anagallis arvensis* on molluscs, *Biomphalaria alexandrina*, was also feasible to reach lethal concentrations, with 50 mg/L being the lethal concentration and 90 mg/L being the lethal concentration (5).

The results show that *Phyllanthus niruri* aqueous stem and leaf extract and *Euphorbia tirucalli* aqueous stem, leaf, and latex extract are poisonous to the freshwater snails *Lymnaea acuminata* and *Indoplanorbis exustus*. Toxicity data from this investigation indicates that the plant extracts mentioned above have significant molluscicidal efficacy. In *Lymnaea acuminata* and *Indoplanorbis exustus*, the extracts generated considerable behavioural changes, with the most visible symptom of discomfort being muscular and spiral twisting of the body, followed by crawling on one another. The type and early commencement of these behavioural reactions indicate that the extracts most likely include neurotoxins, which may be active in the exposed animals' neuromuscular system, among other places. Singh and Agarwal (13) noticed similar behaviour reactions in their laboratory investigation on the acute toxicity of *Euphorbia royleana*, *Euphorbia antisyphiliatica*, and *Euphorbia tirucalli* lattices on snail *Lymnaea acuminata.*

No behavioural symptoms or death were seen in control animals that were not given treated water, showing that the changed behaviour and mortality were caused by something other than plant moieties. The animal's concentration-dependent response might be caused by a number of elements, including penetration rate, slope, variability, and maximum impact.

The LC50 values of the studied aqueous extracts of *Phyllanthus niruri* aqueous stem bark and leaves extract dropped from 267.89 mg DW/L (24h);> to 180.64 mg DW/L(96h) and 229.37 mg DW/L(24h);> to 136.40 mg DW/L(96h) in the case of *Lymnaea acuminata* and *Indoplanorbis exustus.* At all exposure times, the stem bark and latex of *Euphorbia tirucalli* were shown to be harmful to both snails in the same way.

Reduced toxicity might be attributed to soil particle adsorption or temperature-induced acceleration of the toxicant breakdown process. Perschbacher and Sarkar (10) found that the toxicity of *Masea ramentacea* and tea seed cake was short-lived, and fish could be stocked into ponds four days after spraying the plant pesticides.

Increased mortality with longer exposure durations might be caused by a number of reasons, which may work independently or in concert. For example, the updating of the active moiety is time dependent, resulting in a gradual rise in the drug's entry and effects in the snail body. The rate of detoxification in the animal body, as well as the stability (life span) of the active moiety of pesticide in the environment, affect the mortality and exposure periods connection.

The data on toxicity is subjected to a statistical analysis, which reveals numerous key facts. The χ^2 test for goodness of fit (heterogeneity) revealed that the mortality counts were not statistically heterogeneous, and that other factors, such as resistance, did not have a significant impact on the LC_{50} values, which were determined to be within the 95 % confidence limits. The slope is therefore a measure of the target animal's vulnerability to plant molluscicides. Rapid absorption and start of effects are also indicated by a steep slope. Despite the fact that the slope alone is not a highly accurate predictor of toxicological mechanism, it is a valuable metric for this type of research. Since LC_{50} of the aqueous extracts was within the 95% confidence levels, it

is self-evident that the concentration response lines would fall within the same range in a replication test of random samples (11).

Thus aqueous extracts of above plants potent to the control of snails and safe for other non-target organism.

References

1. APHA /AWWA/ WEF (1998). Standard methods for the examination of water and wastewater. 20th edition, American public health association, New York, USA
2. Edilene Carvalho Gomes Ribeiro, José Antonio Costa Leite, Tássio Rômulo Silva Araújo Luz, Daniella Patrícia Brandão Silveira, Samara Araújo Bezerra, Gleycka Cristine Carvalho Gomes Frazão, Luciana Patrícia Lima Alves Pereira, Everton Gomes Guimarães dos Santos, Paulo Roberto Campos Flexa Ribeiro Filho, Alexandra Martins Santos Soares, Ricardo Luvizotto Santos, Denise Fernandes Coutinho (2021). Molluscicidal activity of monoterpenes and their effects on inhibition of acetylcholinesterase activity on *Biomphalaria glabrata*, an intermediate host of *Schistosoma mansoni*, Acta Tropica, Volume 223, 106089,
3. H. Kloos and F. S. McCullough (1982). Plant Molluscicides, Journal of Plant Medicinal, Vol. 46, pp. 195—209
4. Jia, TW., Wang, W., Sun, LP. *et al.* (2019). Molluscicidal effectiveness of Luo-Wei, a novel plant-derived molluscicide, against *Oncomelania hupensis*, *Biomphalaria alexandrina* and *Bulinus truncatus*. *Infect Dis Poverty* 8, 27.
5. Keyla Nunes Farias, José AAdosS, Robson Xavier Faria. (2021). Aqueous Extracts: An Alternative in the Control of the Intermediate Host of Schistosomiasis. - 4(3) OAJBS.ID.000302.

6. Lemma. A. P., Goll J. Duncan and B. Mazengia, 1978: Control of schistosomiasis with use of endod in Adwa, Ethiopia: results of a five year study. Proceedings of the International Conference on Schistosomiasis, Cairo 5. 0. P. Press. Pp. 415-436.
7. Lemma A (1965). Preliminary report on the molluscicidal property of endod (*Phytolacca dodecandra*), Ethiopian Medical Journal, 3. 187
8. Ludevese, F., Ludevese, Pascua, G., and Portugaliza, H. (2018). Molluscicidal activity of *Jatropha curcas* L. crude leaf extracts against *Oncomelania hypensis quadrasi* Mollendorf. Journal of Science, Engineering and Technology, 6: 177-186.
9. Mandefro, B., Mereta, S.T., Tariku, Y. *et al.* (2017). Molluscicidal effect of *Achyranthes aspera* L. (Amaranthaceae) aqueous extract on adult snails of *Biomphalaria pfeifferi* and *Lymnaea natalensis*. *Infect Dis Poverty* **6,** 133
10. Perschbacher, P.W., and Sarkar, J. (1992). Toxicity of selected pesticides to the snakehead, Channa punctata. Asian Fish Sci., (2): 249-254.
11. Rand, G.M., and Petrocelli, S.R. (1988). Fundamentals of aquatic toxicology. Rand, G.M. and Petrocelli, S. R. (Eds.) Hemisphere Publishing, New York, p. 415.
12. Robertson, J.L, Russell R.M, Preisler H.K. Savin, N.E. 2007. Bioassay with Arthropods: POLO computer programme for analysis of bioassay data. 2nd ed. Boca Raton: CRC Press; Pp. 1-224.
13. Singh, A., Agarwal, R.A., (1988). Possibility of using latex of Euphorbiales for snail control. The Sci. Total Environment, 77: 231-236.
14. Sokal, R.R., Rohlf, F.J. (1973). Introduction of Biostatistics. W.H. Freeman and company, San Francisco, pp. 368.

Table 1. Toxicity (LC_{10}, LC_{50} and LC_{90}) of aqueous stem and leaf extract of *Phyllanthus niruri* against *Lymnaea acuminata* at different time intervals.

Exposure periods	Effective dose(W/V) (mg DW/L)	Limit (mg DW/L		Slope value	't' ratio	'g' value	Heterogeneity
		LCL	UCL				
24h	LC_{10}=173.98 LC_{50}=267.89 LC_{90}=412.4	160.40 230.98 310.61	187.40 402.17 924.19	9.11±4.05	3.39	0.012	0.24
48h	LC_{10}=101.03 LC_{50}=225.13 LC_{90}=501.68	59.10 197.22 339.43	120.93 326.02 704.22	3.00±1.35	3.16	0.173	0.19
72h	LC_{10}=80.46 LC_{50}=205.10 LC_{90}=522.78	30.24 181.77 337.39	105.29 294.36 665.00	2.73±1.23	2.83	0.249	0.24
96h	LC_{10}=93.23 LC_{50}=180.64 LC_{90}=350.00	62.73 168.01 277.29	110.67 203.17 605.26	2.75±1.24	3.99	0.238	0.22

- Batches of ten snails were exposed to four different concentrations of aqueous extracts of stem and leaf of *Phyllanthus niruri.*
- Concentrations (Dry weight of stem and leaves) given are the final concentrations W/V in aquarium water.
- Regression coefficient showed that there was significant negative regression between exposure time and different LC values, LCL: lower confidence limit UCL; upper confidence limit.
- There was no mortality in control groups.

Table 2. Toxicity (LC_{10}, LC_{50} and LC_{90}) of aqueous latex extract of *Euphorbia tirucalli* against *Lymnaea acuminata* at different time intervals.

Exposure periods	Effective dose(W/V) (mg DW/L)	Limit (mg DW/L		Slope value	't' ratio	'g' value	Heterogeneity
		LCL	UCL				
24h	LC_{10}=0.65 LC_{50}=1.80 LC_{90}=4.93	0.53 1.26 2.50	0.77 5.01 38.6	0.13± 0.8	3.23	0.011	0.19
48h	LC_{10}=0.47 LC_{50}=1.83 LC_{90}=7.07	0.32 1.25 3.15	0.57 5.26 73.78	0.84±0.42	3.34	0.043	0.25
72h	LC_{10}=0.24 LC_{50}=0.92 LC_{90}=3.45	0.14 0.77 2.09	0.32 1.27 10.22	0.60 ±0.26	4.35	0.138	0.32
96h	LC_{10}=0.16 LC_{50}=0.65 LC_{90}=2.63	0.07 0.56 1.70	0.23 0.79 6.70	0.56 ±0.22	4.41	0.239	0.17

- Batches of ten snails were exposed to four different concentrations of aqueous extracts of latex extract of *Euphorbia tirucalli.*
- Concentrations (Dry weight of latex) given are the final concentrations W/V in aquarium water.
- Regression coefficient showed that there was significant negative regression between exposure time and different LC values, LCL: lower confidence limit. UCL; upper confidence limit.
- There was no mortality in control groups.

Table 3. Toxicity (LC_{10}, LC_{50} and LC_{90}) of aqueous bark extract of *Euphorbia tirucalli* against *Lymnaea acuminata* at different time intervals.

Exposure periods	Effective dose(W/V) (mg DW/L)	Limit (mg DW/L		Slope value	't' ratio	'g' value	Heterogeneity
		LCL	UCL				
24h	LC_{10}=158.75 LC_{50}=239.19 LC_{90}=360.38	140.33 222.97 304.67	169.77 272.65 509.15	5.46±2.39	4.65	0.072	0.59
48h	LC_{10}=134.19 LC_{50}=224.28 LC_{90}=374.84	106.55 209.31 307.66	149.48 255.35 582.97	4.20±1.84	4.22	0.158	0.20
72h	LC_{10}=132.44 LC_{50}=195.88 LC_{90}=289.72	113.67 187.70 260.70	144.54 206.10 349.98	4.10±1.80	5.61	0.191	0.14
96h	LC_{10}=126.26 LC_{50}=179.56 LC_{90}=255.37	109.33 172.01 236.63	137.70 186.66 289.39	4.12±1.81	6.24	0.256	0.11

- Batches of ten snails were exposed to four different concentrations of aqueous extracts of bark extract of *Euphorbia tirucalli.*
- Concentrations (Dry weight of latex) given are the final concentrations W/V in aquarium water.
- Regression coefficient showed that there was significant negative regression between exposure time and different LC values, LCL: Lower confidence limit. UCL: Upper confidence limit.
- There was no mortality in control groups.

Table 4. Toxicity (LC_{10}, LC_{50} and LC_{90}) of aqueous stem and leaf extract of *Phyllanthus niruri* against *Indoplanorbis exustus* at different time intervals.

Exposure periods	Effective dose(W/V) (mg DW/L)	Limit (mg DW/L		Slope value	't' ratio	'g' value	Heterogeneity
		LCL	UCL				
24h	LC_{10}=120.05 LC_{50}=261.49 LC_{90}=569.58	98.45 229.37 422.65	135.65 326.81 999.79	1.18±0.52	5.21	0.057	0.33
48h	LC_{10}=82.18 LC_{50}=210.86 LC_{90}=540.99	58.06 187.91 397.20	99.30 251.76 977.31	0.83±0.37	5.08	0.155	0.15
72h	LC_{10}=65.93 LC_{50}=164.72 LC_{90}=411.50	44.61 149.06 323.24	81.98 184.05 636.52	0.76±0.35	5.44	0.242	0.17
96h	LC_{10}=51.74 LC_{50}=136.40 LC_{90}=359.57	30.87 119.95 286.41	68.12 151.45 547.97	0.75±0.34	5.17	0.341	0.15

- Batches of ten snails were exposed to four different concenterations of aqueous extracts of stem and leaf of *Phyllanthus niruri.*
- Concentrations (Dry weight of stem and leaves) given are the final concentrations W/V in aquarium water.
- Regression coefficient showed that there was significant negative regression between exposure time and different LC values, LCL: Lower confidence limit UCL: Upper confidence limit.
- There was no mortality in control groups.

Table 5. Toxicity (LC_{10}, LC_{50} and LC_{90}) of aqueous stem bark extract of *Euphorbia tirucalli* against *Indoplanorbis exustus* at different time intervals.

Exposure periods	Effective dose(W/V) (mg DW/L)	Limit (mg DW/L		Slope value	't' ratio	'g' value	Heterogeneity
		LCL	UCL				
24h	LC_{10}=143.13 LC_{50}=239.33 LC_{90}=367.44	130.08 199.32 278.86	153.71 320.41 731.45	5.93±2.72	3.79	0.012	0.28
48h	LC_{10}=138.91 LC_{50}=203.95 LC_{90}=299.44	127.58 186.18 248.80	147.09 243.72 440.99	6.19±2.83	4.56	0.009	0.32
72h	LC_{10}=94.33 LC_{50}=170.79 LC_{90}=309.22	76.16 157.88 251.03	105.77 194.55 466.56	2.21±1.03	4.89	0.124	0.26
96h	LC_{10}=94.85 LC_{50}=159.71 LC_{90}=266.66	79.79 149.54 228.09	104.91 173.46 352.40	2.22±1.03	5.60	0.125	0.47

- Batches of ten snails were exposed to four different concentrations of aqueous extracts of stem bark extract of *Euphorbia tirucalli.*
- Concentrations (Dry weight of stem and leaves) given are the final concentrations W/V in aquarium water. Regression coefficient showed that there was significant negative regression between exposure time and different LC values, LCL: lower confidence limit. UCL: upper confidence limit
- There was no mortality in control groups.

Table6. Toxicity (LC_{10}, LC_{50} and LC_{90}) of aqueous latex extract of *Euphorbia tirucalli* against *Indoplanorbis exustus* at different time intervals**.**

Exposure periods	Effective dose(W/V) (mg DW/L)	Limit (mg DW/L		Slope value	't' ratio	'g' value	Heterogeneity
		LCL	UCL				
24h	LC_{10}=0.18 LC_{50}=0.90 LC_{90}=4.39	0.11 0.70 2.39	0.24 1.41 14.27	0.53±0.14	4.99	0.037	0.44
48h	LC_{10}= 0.08 LC_{50}=0.77 LC_{90}=7.45	0.12 0.56 3.19	0.44 1.33 45.90	0.38±0.86	4.24	0.124	0.28
72h	LC_{10}=0.04 LC_{50}=0.51 LC_{90}=6.50	0.01 0.39 2.80	0.07 0.79 45.08	0.34±0.73	4.24	0.206	0.45
96h	LC_{10}=0.02 LC_{50}=0.28 LC_{90}=3.21	0.006 0.21 1.68	0.05 0.36 12.18	0.34±0.70	4.58	0.292	0.70

- Batches of ten snails were exposed to four different concentrations of aqueous extracts of latex extract of *Euphorbia tirucalli*.
- Concentrations (Dry weight of stem and leaves) given are the final concentrations W/V in aquarium water.
- Regression coefficient showed that there was significant negative regression between exposure time and different LC values, LCL: Lower confidence limit UCL: Upper confidence limit.
- There was no mortality in control groups.

Table 7: Percent mortality (mean±SE) of *Lymnaea acuminata* and *Channa punctatus* caused by aqueous extract of stem, root and latex extracts (i.e. 24h LC_{90} of snail) of *Phyllanthus niruri* and *Euphorbia tirucalli* after 24h exposure period.

Plants	**Plant parts**	**Experimental animals**	**Concentration (mgDW/L)(w/v)**	**% Mortality**
Phyllanthus niruri	Stem and leaf	*L.acuminata* *C.punctatus*	412.48 -	..... zero
Euphorbia tirucalli	Stem bark	*L.acuminata* *C.punctatus*	498.84 -	.... zero
Euphorbia tirucalli	Latex	*L.acuminata* *C.punctatus*	4.93 -	.... zero

- Each aquarium contained ten fish (*Channa punctatus*) and ten snails (*Lymnaea acuminata*) in 6L de-chlorinated tap water.

There was no mortality in control group