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Analysis of *Achyranthes aspera* leaf extract and acute toxicity study on fingerlings of Nile tilapia, *Oreochromis niloticus*

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ABSTRACT

Schistosomiasis is a debilitating chronic disease with great socioeconomic and public health impact affecting the poor rural populations who lack access to sanitation, and safe water supply. The high cost of synthetic molluscicides, their toxicity to non-target organisms, and their persistence in the environment have forced the research of plant-derived molluscicides. Although plant molluscicides are cheap, biodegradable, ecofriendly and less toxic to higher animals, unregulated applications could affect non−target organisms. Therefore, ecotoxicological studies are essential to assess the toxicity of these substances to economically and ecologically significant fish species and to establish safe dosage level. This study is intended to investigate the acute toxicity of a molluscicidal plant *Achyranthes aspera* to Nile tilapia fingerlings, *Oreochromis niloticus* (O. niloticus) (n = 7) were exposed to serial dilutions of *A. aspera* leaf aqueous extract using maceration method for 96 h in triplicate setup. Phytoconstituents were identified by GC-MS. Mortality data were analyzed by probit regression to determine lethal concentrations. The NOAEC was ascertained through hypothesis testing based on survival data. The respective piscicidal LC_1 and LC_{10} values were 897.43 and 1063.87 mg/L while the LC_{50} is 1310.74 mg/L. In addition, the NOAEC was 1100 mg/L ($p > 0.05$). This piscicidal toxicity is much lower than its molluscicidal potency may be due to the presence of rotenones and triterpenoides which are commonly found in piscicidal natural products. The GC-MS analysis revealed 12 phytoconstituents including a monoterpene. This study indicates that *A. aspera* has low toxicity to Nile tilapia could be due to monoterpenes are nontoxic. The findings of this study demonstrate that, at this dose, the plant is safe to the test fish. Thus it can be effective, eco-friendly and sustainable alternative for the development of molluscicides for snail control.

1. Introduction

Schistosomiasis, a neglected tropical disease (NTD), is one of the most important prevalent human and animal parasitoses in tropical and subtropical region worldwide [1]. Schistosomiasis is a debilitating chronic disease with great socioeconomic and public health impact affecting the poor rural populations who lack access to sanitation, and safe water supply [2]. In this regards, snail control plays a central role in schistosomiasis [3]. The applicability of the available synthetic molluscicide (niclosamide) is challenging due to high cost and environmental concern.

Although it is a great challenge to control schistosomiasis in undeveloped regions because of financial burden and environmental pressures [3]. Therefore, the current strategies for schistosomiasis control mainly rely on snail control [4]. Thus forced researchers look for alternative botanical products. Ease of access at low cost and environmental friendliness is the major attributes of many medicinal plants. Similar refreshment is being observed in the search for molluscicidal plants against vector snails transmitting schistosomiasis and other trematode parasites [5–7].

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Plant molluscicides are generally considered as eco-friendly, rapidly biodegradable and less toxic to higher animals. Some plant molluscicides are considered inexpensive and environmental friendly to the health of aquatic organisms and mammals, and some pure compounds have also been isolated from plant and proven to have molluscicidal activity against snails [1]. Development of natural plant molluscicides may be a suitable alternative for snail control [8,9]. However, their indiscriminate use could affect nontarget organisms. The toxic effects of these products, especially on important species like fish, should be investigated before widespread use. Such ecotoxicological information also fosters their practical applicability [10,11]. Acute fish toxicity has been assessed for only few molluscicidal plants like *Phytolacca dodecandra* [12], *Jatropha curcas* [13] and *Moringa oleifera* [10,14].

Beside toxicity studies, knowledge of the chemical constituents of such plants is desirable to develop a more comprehensive information regarding its toxicological potential, safety and efficacy [15]. In addition, identification and validation of the bioactive compounds is useful for further understanding of its molluscicidal properties and for the synthesis of effective chemical substances [16].

Achyranthes aspera (*A. aspera*), a member of the Amaranthaceae family, is a perennial herb growing in many parts of Asia, Africa, America, Europe and Australia [17,18]. It is a well-known medicinal plant in Ethiopia, India and other countries [19,20]. *A. aspera* is traditionally popular in folk remedy in tropical Asia and in different African countries. It's used in various traditional health care systems including the treatment of fever, wound healing, tooth ache, arthritis, gynecological disorders, urinary disorders, insect and snake bites, abdominal tumor, stomach pain and elsewhere for a number of ailments treatment [21–24]. In east Africa the plant is used for treating tonsillitis, head wounds and ringworm. Different plant parts (root, stem, leaf, inflorescence, and seeds) are used individually for treating different illnesses, though the whole plant is also often used [25,26].

Studies reported that *Achyranthes aspera* extract can be used as antifungal, antibacterial, antioxidant, antifertility and in the treatment of renal dropsy, skin rash, chronic malaria, impotence, asthma, diabetes etc [27–29]. Recently, the molluscicidal properties of *A. aspera* have been discovered [24]. Phytochemical studies on *A. aspera* revealed presence of flavonoids, alkaloids, saponins and cardiac glycosides [29–31]. Saponins and alkaloids are molluscicidal; also, piscicidal.

Nile tilapia is an African freshwater cichlid fish naturally occurring in rivers, dams and lakes as an important ecological entity. It is an important food fish all over the world and is most exploited species constituting 60–80 % total fish capture [32–35]. Moreover, it is one of the predominantly stocked fish in aquacultures, artificial lakes, reservoirs and small water bodies. Yet, the action of *A. aspera* on those economically important fingerlings of Nile tilapia, *Oreochromis niloticus* is not known so far.Therefore, the plant's molluscicidal application requires further phytochemical analysis and ecotoxicological investigations. Herein, this study is aimed to investigate acute toxicity of *A. aspera* leaves aqueous extract to *Nile tilapia* fish, *O. niloticus* and identify major phytoconstituents.

2. Materials and methods

A standard non-renewed static test was adopted for this test to evaluate the fish acute toxicity of *A. aspera* leaves aqueous extract in 96 h exposure time.

2.1. Plant material collection and processing

In Ethiopia, *A. aspera* grows abundantly as a weed on abandoned lands. Verbal consent for this research was obtained from the district agricultural office. Fresh leaves were collected from a natural habitat at 9°43′45.59″ N, 39°37′2.71″ E in Keyit district, North Shewa, Oromia, Ethiopia. Specimen was kept in Addis Ababa University Herbarium with number: M.B.1. It was dried in shade and powdered to 200 μm particle size.

For each serial dilution, crude aqueous extraction was made using maceration method as described by Ref. [36] with slight modification. The required amount of the plant powder was placed in 400 mL aged water in a conical flask and shaking at 160 rpm, overnight. Finally, it was filtered using a clean cotton cloth and stored at 4 °C until used [37].

2.2. Test animal collection and maintenance

110 fingerlings of *O. niloticus* were taken by permission from an artificial fishpond owned by Department of Biology, Dilla University, Ethiopia. Fingerlings of either sex were evenly distributed into the test concentrations at equal chance, measuring 6.27 ± 0.2 cm in length, and mass of 3.89 ± 0.25 g were selected for this test and the remaining was immediately returned in to the pond [38].

Caught fish were promptly put into clean plastic buckets containing the pond water and immediately brought to the Biology laboratory for acclimatization. During acclimatization the physicochemical properties of water were maintained at temperature (21 °C), dissolved oxygen (75 %), pH (7.8) and total hardness (85 mg/L as $CaCO_3$) were used. They were maintained in two 80 cm × 60 cm x 60 cm aquaria for one week in aged tap water under continuous aeration and 12-h light, 12-h dark photoperiod. They were fed with flakes of tasty soya (Pramukh Agro-industry PLC, Ethiopia) as recommended by Ref. [39]. Feeding was stopped 24 h before the experiment [40,41]. In the course of the experiment, the fingerlings of *O. niloticus* were ethically handled in accordance with the principles of animal welfare in scientific experiments.

2.3. Fish acute toxicity test

The range finding test was set according to the protocols defined by OECD [42] and EPA [43]. Five serial dilutions of 100, 400, 800, 1200 and 1600 mg/L aqueous extract were prepared in 10 L aged water. Three randomly selected healthy fish were exposed to each test solution for 24 h. The purpose of the range finding test is to identify the useful concentration range that would probably produce mortality rates between 0 and 100 % and guess the range of concentrations for the definitive test. Initially, a range−finding test was conducted. As a result, six dilutions: 600, 800, 1000, 1100, 1200 and 1400 mg/L, each in 30 L aged tap water, were prepared for the definitive test. According to the protocols [42,44,45], seven healthy fishes of either sex were randomly placed and exposed to each test solution for 96-h being intermittently aerated in every 2–3 h. Aged tap water was used for negative control. The test was prepared in triplicates. Mortalities were recorded at the end of 12, 24, 48, 72 and 96 h [42]. Dead fish were identified by absence of tail and gill movements and loss of sensation to gentle prodding with a glass rod [46]. Dead fish were immediately picked out from the solutions to minimize contamination. Surviving fish were euthanized by immersion in 40 L 0.4 mL/L clove oil solution according to AVMA guideline [47,48].

2.4. Water physicochemical characteristics

Dissolved oxygen, temperature and pH were measured in every 24 h using a calibrated multiparameter probe. Before every measurement, the probe was calibrated according to its operating manual. Total hardness was determined only at the start by EDTA titrimetric method using Eriochrome Black T (EBT) as indicator and calculated using the described equation below.

Hardness in mg/L as $CaCO_3$=(V x N x 50 x 1000)/(SV)

Where: V = volume of titrant (mL); N = normality of EDTA; 50 = equivalent weight of $CaCO_3$; SV = sample volume (mL)

2.5. Phytochemical analysis of A. aspera by GC-MS

The phytochemical profile of *A. aspera* leaf crude ethanolic extract was carried out using the procedure described by Olivia, N.U. et al. [49]. Briefly, the GC-MS analysis was carried out in a combined 7890A gas chromatograph system (Agilent 19091-433HP, USA) and mass spectrophotometer, fitted with a HP-5 MS fused silica column (5 % phenyl methyl siloxane 30.0 m × 250 μm, film thickness 0.25 μm), interfaced with 5675C Inert MSD with Triple-Axis detector. Helium gas was used as carrier gas and was adjusted to column velocity flow of 1.0 ml/min. Other GC-MS conditions are ion-source temperature, 250 °C; interface temperature, 300 °C; pressure, 16.2 psi; out time, 1.8 mm; and 1 μl injector in split mode with split ratio 1:50 with injection temperature of 300 °C. The column temperature started at 40 °C for 5 min and changed to 150 V at the rate of 4 °C/min. The temperature was raised to 100 °C at the rate of 20 °C/min and held for 5 min and from 100 to 310 °C at 10 °C/min while initial and final holdup time was 1 and 16 min, respectively. The temperature of the injector and MS transfer line were set at 250 and 280 °C, respectively. The total elution was 47.5 min. The relative percent amount of each component was calculated by comparing its average peak area to total areas. MS solution software provided by supplier was used to control the system and to acquire the data. An electron ionization system (with ionization energy of 70eV) was used for GC-MS detection while the scanning mass was ranged from 33 to 500 *m/z*.

2.6. Data analysis

The phytoconstituents were identified by comparing the spectra of each unknown compounds with that of known compounds archived in a database repository. Effective doses (LC_1, LC_{10} and LC_{50}) were determined from the mortality data by probit regression method in IBM SPSS software. The NOAEC (No observed adverse effect concentration) is by definition, the highest concentration at which survival of the test organism is not significantly different from the control [43,50]. Therefore, the NOAEC was determined by hypothesis testing using Dunnett *t*-test at 95 % confidence interval [43].

First: the mortality rates are recorded as response proportion data for each concentration and control group. (RP) = Number of survivors/Total number of exposed.

Where RP is response proportion.

Second: The resulting RPs are transformed to arc sine values in radian as follows.

(1) For RPs greater than zero or less than one

$$\text{Angle (in radians)} = arc\ sine\sqrt{(RP)}$$

(2) Modification of the arc sine when RP = 0.

$$\text{Angle (in radians)} = \text{arc sine}\ \sqrt{1/4n}$$

Where n = number of animals per treatment replica.

(3) Modification of the arc sine when RP = 1.0.

$$\text{Angle} = 1.5708\ \text{radians} - (\text{radians for RP} = 0)$$

Third: Dunnett test is performed on the arc sine transformed mortality data at 95 % confidence interval to determine the concentration that exhibit statistically insignificant mortality difference from the control.

3. Results

3.1. Bench side observation

The fingerlings remained very active in swimming and feeding in the course of acclimatization. No fish has died in the aquaria and no one has exhibited any abnormal operculum beating as signs of stress or suffocation. In few moments following loading of fish to the aquaria, few fingerlings jumped out but were promptly picked up and returned. Afterwards, they became calm and relaxed within 2–3 h. Unlike the control groups, fish exposed to the test solutions are constantly coming to the air water interface and continuously gulp. They usually come to the surface and gulp in groups or rest at the bottom unlike the control groups which were freely moving and chasing each other.

3.2. Water physicochemical properties

In every measurement, dissolved oxygen level was above 60 % however, it was lower in the test solutions than the control. The physicochemical properties such as temperature (℃), dissolved oxygen (%), pH and total hardness (mg/L as CaCO3) at different measurement time are presented in Table 1. The pH level decreased over the days yet its sstandard deviation was below the 1.5 limit for a valid test [42].

3.3. Fish acute toxicity test results

The majority of fish mortalities occurred within the first 48 h (Table 2). No fish has died in the control, 600 mg/L and 800 mg/L dilutions. Dying fish gradually become sluggish, unable to escape when touched. They continuously swirl about and finally sank down.

Concentrations of LC_1, LC_{10} and LC_{50} with respective confidence limits were estimated from the mortality data by probit analysis

Table 1
Measurements of some physicochemical characteristics of test water in fish acute toxicity test.

Measurement hours	Test groups	Test conc. (mg/L)	Temp. (℃)	Dissolved oxygen (%)	pH	Total hardness (mg/L as $CaCO_3$)
24 h	Control	0	21	75	7.8	85
	T.S. 1	600	21	65	7.6	89
	T.S. 2	800	21.2	69	7.8	85
	T.S. 3	1000	21.2	71	7.5	85
	T.S. 4	1100	21.5	67	7.7	86
	T.S. 5	1200	21.5	64	7.6	82
	T.S. 6	1400	20.4	73	7.2	86
48 h	Control	0	20.8	80	7.2	
	T.S. 1	600	20.6	61	7	
	T.S. 2	800	20.5	64	7	
	T.S. 3	1000	20.6	66	7	
	T.S. 4	1100	20.5	60	7	
	T.S. 5	1200	20.6	61	7	
	T.S. 6	1400	20.6	61	7.1	
72 h	Control	0	20.9	76	7	
	T.S. 1	600	20.7	60	7	
	T.S. 2	800	20.8	61	7	
	T.S. 3	1000	20.8	62	7	
	T.S. 4	1100	20.9	68	7	
	T.S. 5	1200	21.3	61	7	
	T.S. 6	1400	20.4	69	7.2	
96 h	Control	0	21.4	78	7	
	T.S. 1	600	21.2	64	7	
	T.S. 2	800	21.1	76	7	
	T.S. 3	1000	21.2	72	7	
	T.S. 4	1100	21.1	70	7	
	T.S. 5	1200	20.9	73	7.1	
	T.S. 6	1400	20.4	70	7.2	
Mean			20.87	67.75	7.18	85.43
SD			0.38	6.01	0.27	2.07

Table 2
Cumulative mortalities of *O. niloticus* fish fingerlings in different extract concentrations during 24, 48, 72 and 96 h of exposure.

Test conc. (mg/L)	Number of fish exposed	Fish cumulative mortalities at different time intervals			
		24 h	48 h	72 h	96 h
0 (Control)	14	0	0	0	0
600	14	0	0	0	0
800	14	0	0	0	0
1000	14	0	0	0	1
1100	14	0	1	1	1
1200	14	3	5	5	5
1400	14	3	6	7	9

Table 3
Lethal concentrations and confidence intervals resulted from probit analysis.

Lethal concentrations	Concentration (in mg/L)	Confidence interval (at 95 %)	χ2
LC1	897.43	642.48–1006.01	1.02
LC10	1063.87	903.01–1140.89	
LC50	1310.74	1229.53–1484.42	

(Table 3). The calculated χ^2 at 95 % confidence interval asserts it's goodness-of-fit.

The response proportions (RP) and corresponding arc sine transformed values in each test solutions are summarized in Table 4. The result indicated the highest concentration in which survival of the fingerlings is not significantly different from the control is the 1100 mg/L ($p > 0.05$). This level is therefore, the NOAEC (No−Observed−Adverse−Effect−Concentration).

3.4. Phytochemical analysis of A. aspera leaf ethanolic extract

The leaf powder was extracted using 85 % ethanol through a microwave-assisted extraction technique. The resulting extract was analyzed by GC-MS and revealed 12 major phytoconstituents (Fig. 1).

4. Discussion

A. aspera is a well-known medicinal plant traditionally used for ailments of various diseases in many parts of the world [19,51]. In addition, the molluscicidal potential of this plant is recently discovered [52]. But assessment on its negative toxic impact on non-target species is mandatory before applied in the aquatic environment for snail control.

Here, the toxic effect of *A. aspera* leaf aqueous extract on an important fish species, *O. niloticus* fingerlings, was studied to evaluate the possible adverse impact on survival of such non-target and important species in case of its application against aquatic vector snails. As a result, the NOAEC was determined to be 1100 mg/L ($p > 0.05$) and the LC_{50} was 1310.74 mg/L. In addition, the respective LC_1 and LC_{10} values were 897.43 mg/L and 1063.87 mg/L.

Fish toxicity effects of different molluscicidal plants such as *Sapindus mukorossi* exhibited an LC_{50} of 10 ppm while its molluscicidal LC_{50} is 119.57 ppm [53], *Jatropha gossypifolia* had piscicidal LC_{50} of 10.490 mg/L [11] while its molluscicidal LC_{50} is over 100 ppm [12,54,55]. The piscicidal LC_{50} of Endod (*Phytolacca dodecandra*) is 4.4 mg/L and its molluscicidal LC_{50} is 10 ppm [12,55]. Similarly, *Carica papaya* exhibited piscicidal LC_{50} of 700 ppm against *O. mossambicus* fish while its molluscicidal LC_{50} is from 619.1 to 2716.3 ppm [56]. These data indicate the molluscicidal medium lethal dose (LC_{50}) of each plant exceeds its respective piscicidal LC_{50}. And hence, they are more toxic to those nontarget fish than to the target snails. Such problem in selectivity and target specificity limits application of these natural products for snail control in habitats where fishes and snails co-exist.

On the contrary, the current study showed that the piscicidal LC_{50} of *A. aspera* leaf aqueous extract to Nile tilapia fingerlings is 1310.74 mg/L and the NOAEC level is 1100 mg/L. These concentrations are much higher than its molluscicidal LC_{50} which is 72.4 ppm, according to Mandefro et al., [24]. This shows a considerable gap between the molluscicidal and piscicidal lethal doses of *A. aspera*. This indicates the plant has minimum toxic effect to this economically and ecologically valuable fish, O. niloticus, especially at its molluscicidal dose limits. This selectivity fosters the plant's applicability for snail control in habitats where fishes and snails co-exist.

Several phytochemical studies on the same plant have identified different classes of saponins [17,57,58]. But in the current GC-MS analysis, saponins are not detected (Fig. 1). However, some compounds identified are molluscicidal by nature. For example, *cis*-p-mentha-1(7), 8-dien-2-ol is an oxygenated monoterpene, a terpenoid saponin moiety [59]. In addition, the larvicidal property of eicosanoic acids and phthalates is indicated in many literatures. The molluscicidal effect of terpenoids is documented in numerous studies [10,60]. In general, the bioactivity of crude extracts often results from the synergistic effects of its constituents.

In all physicochemical measurements, lower dissolved oxygen (DO) level was recorded in test solutions than in the control. The decrease in DO of the test solutions was within the suggested tolerance and so could not have affected the mortality of the test fish [46]. This phenomenon agrees with the studies of Ayuba et al. [46], and Ojutiku et al., [61]. Biodegradation of the phytochemicals leads to higher biological and chemical oxygen demand and causes depletion of DO in the test solution [43,61].

Fingerlings in the test solution were seen motionless resting at the bottom or incessantly gulping at the water air interface. These behavioural changes also happened in many similar studies [11,40,62]. The reactions could be due to dissolved oxygen depletion by the chemicals leading to oxidative stress [61]. It can also be a manifestation of neuro toxicity and poisoning of the gills by the toxicants [61,62].

5. Conclusion

As a general principle, molluscicides are directly applied to snail-infested water bodies where many other non-target species live together. As a result, they are always subjected to deleterious toxicity by such chemicals applied in to their common habitats. To mitigate such

Table 4
Arc sine transformation of survival proportion data.

	Replicate	RP in test concentrations (mg/L) and the control						
		Control	600	800	1000	1100	1200	1400
Raw data	A	1.0	1.0	1.0	1.0	1.0	0.7	0.4
	B	1.0	1.0	1.0	0.9	0.9	0.6	0.3
Arc sine value	A	1.3807	1.3807	1.3807	1.3807	1.3807	1.0069	0.7137
	B	1.3807	1.3807	1.3807	1.1832	1.1832	0.8531	0.5640
	Mean	1.3807	1.3807	1.3807	1.2820	1.2820	0.9300	0.6389
	Var.	0	0	0	0.0195	0.0195	0.0118	0.0112

Note: RP is response proportions.

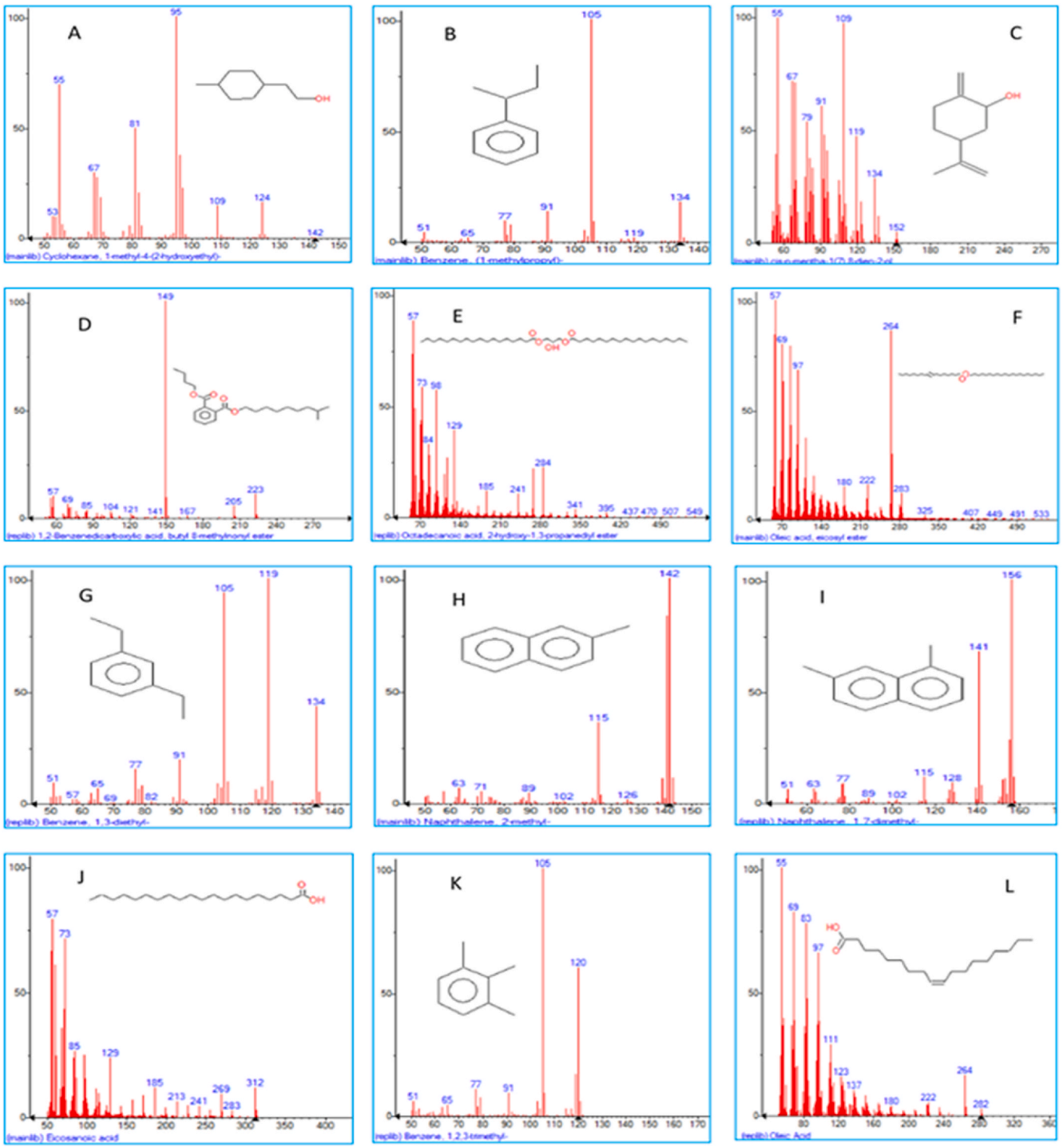

Fig. 1. Phytoconstituents of A. aspera leaf ethanolic extract analyzed by GC-MS. (a) Cyclohexane, 1-methyi-4-(2-hydroxyethyl) $C_9H_{18}O$; (b) Benzene, (1-methyl-propyl) $C_{10}H_{14}$; (c) *Cis*-p-mentha-1(7),8-dien-2-ol $C_{10}H_{16}O$; (d) 1,2-Benzenedicarboxilic acid, butyl 8-methylnonyl ester $C_{22}H_{34}O_4$; (e) Octadecanoic acid, 2-hydroxy-1,3-propanediylester $C_{39}H_{76}O_5$; (f) Oleic acid, eicosyl eter $C_{38}H_{74}O_2$; (g) Benzene,1,3-diethyl $C_{10}H_{14}$; (h) Naphthalene, 2-methyl $C_{11}H_{10}$; (i) Naphthalene,1,7-dimethyl $C_{12}H_{12}$; (j) Eicosanoic acid $C_{20}H_{40}O_2$; (k) Benzene, 1,2,3-trimethyl C_9H_{12}; (l) Oleic acid $C_{18}H_{34}O_2$.

ecological damage, the molluscicide should be selective in action or the dose limit applied for snail control is proved to be safe to such non-target species. The general notion of biodegradability, target specificity and low mammalian toxicity of plant derived molluscicides does not guarantee their safe and indiscriminate usage. Their toxicity to at least those important non-target groups and the safe dose level to be applied should be known beforehand. The application of phytochemicals in the field can be better justified when molluscicidal efficacy studies are backed by ecotoxicological data. This particular study showed that the toxicity of *A. aspera* aqueous extract to *O. niloticus* fingerlings is very much lower than its molluscicidal efficacy. Therefore, it is possible to conclude that application of the plant product for snail control at the specified LC_{50} or LC_{90} dose is safe to this fish. The findings of this study demonstrate that, at this dose, the plant is safe to the test fish. Thus it can be effective,

economical, eco-friendly and sustainable alternative for the development of molluscicides for snail control. However, further studies on non−target groups such as aquatic invertebrates and mammals should be conducted to generate more complete ecotoxicological information. Furthermore, long-term study is required to assess the potential chronic effects of A. aspera on aquatic organisms.

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Availability of data and materials

All data generated or analyzed during this study are included in this article.

Ethics approval

The internal review board (IRB) of college of Public Health, Jimma University, has issued ethical clearance for this study.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Belayhun Mandefro: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Workineh Mengesha Fereja:** Conceptualization, Data curation, Investigation, Methodology, Resources, Validation, Writing - review & editing. **Dawit Fremichael:** Data curation, Writing - review & editing, Formal analysis. **Seid Tiku Mereta:** Conceptualization, Data curation, Supervision, Validation, Visualization, Writing - review & editing. **Argaw Ambelu:** Conceptualization, Data curation, Supervision, Validation, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

[1] L. Zheng, L. Deng, Y. Zhong, Y. Wang, W. Guo, X. Fan, Molluscicides against the snail-intermediate host of Schistosoma: a review, Parasitol. Res. (2021) 1–39.
[2] Q. Xue, Y. Deng, Y. Liu, Y. Wang, W. Hu, Y. Huang, K. Yang, A retrospective analysis of schistosomiasis related literature from 2011-2020: focusing on the next decade, Acta Trop. 238 (2023), 106750.
[3] E.C.G. Ribeiro, J.A.C. Leite, T.R.S.A. Luz, D.P.B. Silveira, S.A. Bezerra, G.C.C. G. Frazão, L.P.L.A. Pereira, E.G.G. Dos Santos, P.R.C.F. Ribeiro Filho, A.M. S. Soares, Molluscicidal activity of monoterpenes and their effects on inhibition of acetylcholinesterase activity on Biomphalaria glabrata, an intermediate host of Schistosoma mansoni, Acta Trop. 223 (2021), 106089.
[4] Y. Limpanont, O. Phuphisut, O. Reamtong, P. Adisakwattana, Recent advances in Schistosoma mekongi ecology, transcriptomics and proteomics of relevance to snail control, Acta Trop. 202 (2020), 105244.
[5] T. Angaye, S.E. Bassey, E.I. Ohimain, S.C. Izah, P.I. Asaigbe, Molluscicidal and synergicidal activities of the leaves of four Niger delta mangrove plants against schistosomiasis vectors, J. Environ. Treat. Tech. 3 (2015) 35–40.
[6] R. de Carvalho Augusto, N. Merad, A. Rognon, B. Gourbal, C. Bertrand, N. Djabou, D. Duval, Molluscicidal and parasiticidal activities of Eryngium triquetrum essential oil on Schistosoma mansoni and its intermediate snail host Biomphalaria glabrata, a double impact, Parasites Vectors 13 (2020) 1–11.
[7] B. Otarigho, O.A. Morenikeji, Molluscicidal effects of aqueous and ethanolic extracts of Lemongrass (Cymbopogon citratus) leaf against the different developmental stages of Biomphalaria pfeifferi, NY Sci. J. 5 (2012) 70–77.
[8] M.A. Radwan, A.F. Gad, Essential oils and their components as promising approach for gastropod mollusc control: a review, J. Plant Dis. Prot. 128 (2021) 923–949.
[9] G. Zhu, Y. Guan, W. Yang, Y. Lu, Experimental study and application of molluscicidal constituents and mechanisms in plants, Zhongguo Meijie Shengwuxue ji Kongzhi Zazhi= Chin. J. Vector Biol. Control 21 (2010) 509–511.
[10] C.A. Rocha-Filho, L.P. Albuquerque, L.R. Silva, P.C. Silva, L.C. Coelho, D. M. Navarro, M.C. Albuquerque, A.M.M. Melo, T.H. Napoleão, E.V. Pontual, Assessment of toxicity of Moringa oleifera flower extract to Biomphalaria glabrata, Schistosoma mansoni and Artemia salina, Chemosphere 132 (2015) 188–192.
[11] A. Singh, D. Singh, T. Misra, R. Agarwal, Molluscicides of plant origin, Biol. Agric. Hortic. 13 (1996) 205–252.
[12] J. Lambert, J. Temmink, J. Marquis, R. Parkhurst, C.B. Lugt, E. Lemmich, L. Wolde-Yohannes, D. De Savigny, Endod: safety evaluation of a plant molluscicide, Regul. Toxicol. Pharmacol. 14 (1991) 189–201.
[13] M. Rug, A. Ruppel, Toxic activities of the plant Jatropha curcas against intermediate snail hosts and larvae of schistosomes, Trop. Med. Int. Health 5 (2000) 423–430.
[14] E. Ayotunde, O. Fagbenro, O. Adebayo, Toxicity of aqueous extract of Moringa oleifera seed powder to Nile tilapia Oreochromis niloticus (LINNE I779), fingerlings, Int. Res. J. Agric. Sci. Soil Sci. 1 (2011) 142–150.
[15] P. Vivekraj, S. Vinotha, A. Vijayan, V.A. Gideon, Preliminary phytochemical screening and GC-MS analysis of methanolic extract of Turnera subulata Smith (Passifloraceae), J. Pharmaceut. 6 (2017) 174–177.
[16] V. Nandagopalan, M.J. Gritto, A. Doss, GC-MS analysis of bioactive components of the methanol extract of Hibiscus tiliaceus Linn, Asian J. Plant Sci. Res. 5 (2015) 6–10.
[17] A. Sharma, S. Kumar, P. Tripathi, Impact of Achyranthes aspera leaf and stem extracts on the survival, morphology and behaviour of an Indian strain of dengue vector, Aedes aegypti L.(Diptera: Culicidae), J. Mosq. Res. 5 (2015) 1–9.
[18] M.A. Eshete, Z. Asfaw, E. Kelbessa, A review on taxonomic and use diversity of the family Amaranthaceae in Ethiopia, J. Med. Plants Sci. 4 (2016) 185–194.
[19] A. Habtamu, Y. Mekonnen, Evaluation of the antibacterial activities of leaf extracts of Achyranthus aspera, Afri. J. Bacteriology Res. 9 (2017) 9–14.
[20] C.V. Reddy, A. Kamble, Toxicity Study of Achyranthus Aspera, International letters of natural sciences, 2014.
[21] A.R. Ndhlala, H.M. Ghebrehiwot, B. Ncube, A.O. Aremu, J. Gruz, M. Šubrtová, K. Doležal, C.P. du Plooy, H.A. Abdelgadir, J. Van Staden, Antimicrobial, anthelmintic activities and characterisation of functional phenolic acids of Achyranthes aspera Linn.: a medicinal plant used for the treatment of wounds and ringworm in east Africa, Front. Pharmacol. 6 (2015) 274.
[22] S.R. Neeta, B. Jyoti, S. Anjuvan, K. Prabhjot, Antibacterial potential of achyranthus aspera linn procured from Himachal Pradesh, Punjab and Haryana, India, Res. J. Chem. Sci. ISSN 2231 (2011) 606X.
[23] E.C.G. Ribeiro, Moluscicida a partir do óleo essencial de Hyptis pectinata e de monoterpenos identificados em espécies aromáticas nativas da Amazônia, 2021.
[24] B. Mandefro, S.T. Mereta, Y. Tariku, A. Ambelu, Molluscicidal effect of Achyranthes aspera L.(Amaranthaceae) aqueous extract on adult snails of Biomphalaria pfeifferi and Lymnaea natalensis, Infect. Dis. Poverty 6 (2017) 52–56.
[25] C. Shendkar, P. Chandrachood, A. Pawar, S. Lavate, N.R. Deshpande, Quantitative estimation of macro, micro nutrients and trace elements by X-ray fluorescence spectroscopy (XRF) from Achyranthes aspera Linn, 0-963, Magnesium 9630 (2011).
[26] S.S. Dangi, M. Gupta, D. Maurya, V.P. Yadav, R.P. Panda, G. Singh, N.H. Mohan, S. K. Bhure, B.C. Das, S. Bag, Expression profile of HSP genes during different seasons in goats (Capra hircus), Trop. Anim. Health Prod. 44 (2012) 1905–1912.
[27] L.H. Reddy, J.L. Arias, J. Nicolas, P. Couvreur, Magnetic nanoparticles: design and characterization, toxicity and biocompatibility, pharmaceutical and biomedical applications, Chem. Rev. 112 (2012) 5818–5878.
[28] H.S. Mekonnen, M.H. Gebrie, K.H. Eyasu, A.A. Gelagay, Drug adherence for antihypertensive medications and its determinants among adult hypertensive

patients attending in chronic clinics of referral hospitals in Northwest Ethiopia, BMC Pharmacol. Toxicol. 18 (2017) 1–10.
[29] R.D. Reddy, P.N. Reddy, N. Prathibha, T.M. Mounica, A. Phanikumar, M. Ravindra, Pharmacognostic, preliminary phyto chemical and pharmacological studies on the roots of achyranthes aspera, Int. J. Phcog. Phytochem. Res. 4 (2012) 139–141.
[30] A. Dey, Achyranthes aspera L: phytochemical and pharmacological aspects, Int. J. Pharmaceut. Sci. Rev. Res. 9 (2011) 72–82.
[31] V. Kumar, A. Sharma, A.K. Thukral, R. Bhardwaj, Phytochemical profiling of methanolic extracts of medicinal plants using GC-MS, Int. J. Res. Dev. Pharm. Life Sci. 5 (2016) 2153–2158.
[32] M.-H. Breuil, Foreword. On Art and Nature–Composite Collections, CeROArt. Conservation, Exposition, Restauration d'Objets d'Art, Association CeROArt asbl, 2013.
[33] M. Endebu, A. Lema, T. Genet, A. Mitike, B. Regassa, E. Dejen, H. Abegaz, Fisheries baseline survey describing status of fisheries in Lake Zeway, Ethiopia, J .Fish. Livest Prod. 3 (2015) 100–129.
[34] A.M. Janko, Fish production, consumption and management in Ethiopia, Int. J. Econ. Manage. 3 (2014) 1–6.
[35] A. Tesfahun, M. Temesgen, Food and feeding habits of Nile tilapia Oreochromis niloticus (L.) in Ethiopian water bodies: a review, Int. J. Fish. Aquatic Stud. 6 (2018) 43–47.
[36] J. Ismael, E. Dessalegn, W.M. Fereja, In Vitro antioxidant and antibacterial activity of leaf extracts of Measa lanceolata, Int. J. Food Prop. 24 (2021) 702–712.
[37] G.G. Syngai, R. Gopi, R. Bharali, S. Dey, G.A. Lakshmanan, G. Ahmed, Probiotics-the versatile functional food ingredients, J. Food Sci. Technol. 53 (2016) 921–933.
[38] A.A. Mourad, S.L. Bahna, Hypersensitivities to non-steroidal anti-inflammatory drugs, Expet Rev. Clin. Immunol. 10 (2014) 1263–1268.
[39] M.P. Kushwaha, Replacement of fish meal by soybean (Glycine max) in the formulation of fish feed ingredients essential for immunostimulation and growth performance of carps, Int. J. Fauna Biological Stud. 1 (2013) 35–38.
[40] I. Okey, R. Keremah, G. Ikpi, L. Ebeniro, Toxicity of leaf powder of Lepidagathis alopecuroides to Nile tilapia Oreochromis niloticus juveniles, Afr. J. Food Sci. 7 (2013) 128–133.
[41] G.G. Syngai, S. Dey, R. Bharali, Evaluation of toxicity levels of the aqueous extract of Allium sativum and its effects on the behavior of juvenile common carp (Cyprinus carpio L., 1758), Asian J. Pharmaceut. Clin. Res. (2016) 417–421.
[42] F. Busquet, R. Strecker, J.M. Rawlings, S.E. Belanger, T. Braunbeck, G.J. Carr, P. Cenijn, P. Fochtman, A. Gourmelon, N. Hübler, OECD validation study to assess intra-and inter-laboratory reproducibility of the zebrafish embryo toxicity test for acute aquatic toxicity testing, Regul. Toxicol. Pharmacol. 69 (2014) 496–511.
[43] F. Edition, Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, US Environmental Protection Agency US EPA, Washington, DC, USA, 2002.
[44] U. EPA, Ecological effects test guidelines, Gammarid acute Toxic. test OPPTS 850 (1996).
[45] A. Mourad, Molluscicidal Effect of Some Plant Extracts against Two Land Snail Species, Monacha obstructa and Eobania vermiculata, 2019.
[46] V. Ayuba, P. Ofojekwu, S. Musa, Acute toxicity of Clarias gariepinus exposed to Datura innoxia leaf extract, J. Med. Plants Res. 6 (2012) 2453–2457.
[47] W. Underwood, R. Anthony, AVMA Guidelines for the Euthanasia of Animals: 2020 Edition, Retrieved on March 2013 (2020) 2020-2021..
[48] I. Fernandes, Y. Bastos, D. Barreto, L. Lourenço, J. Penha, A eficiência do óleo de cravo como anestésico e em procedimentos de eutanaisa em peixes de pequeno porte, Braz. J. Biol. 77 (2017) 444–450.
[49] N.U. Olivia, U.C. Goodness, O.M. Obinna, Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of Hibiscus asper leaves, Future J. Pharmaceut. Sci. 7 (2021) 1–5.
[50] P.J. Pereyra, G.B. Rossini, G. Darrigran, Toxicity of neem's oil, a potential biocide against the invasive mussel Limnoperna fortunei (Dunker 1857), An Acad. Bras Ciências 84 (2012) 1065–1071.
[51] M. Ayyanar, S. Ignacimuthu, Medicinal Uses and Pharmacological Actions of Five Commonly Used Indian Medicinal Plants: a Mini-Review, 2008.
[52] B. Mandefro, S.T. Mereta, A. Ambelu, Efficacy of Achyranthes aspera (L.) as a Molluscicidal Bait Formulation against Fresh Water Snail Biomphalaria Pfeifferi, Evidence-Based Complementary and Alternative Medicine 2018, 2018.
[53] A. Upadhyay, D. Singh, Pharmacological Effects of Sapindus Mukorossi, vol. 54, Revista do Instituto de Medicina Tropical de São Paulo, 2012, pp. 273–280.
[54] A.A. Pereira Filho, C.R.C. França, D.s.d.S. Oliveira, R.J.d.A. Mendes, J.d.R. S. Gonçalves, I.G. Rosa, Evaluation of the Molluscicidal Potential of Hydroalcoholic Extracts of Jatropha Gossypiifolia Linnaeus, 1753 on Biomphalaria glabrata (Say, 1818), vol. 56, Revista do Instituto de Medicina Tropical de São Paulo, 2014, pp. 505–510.
[55] E.M. Belete, Schistosomiasis control strategies, with emphasis on snail control using molluscicides, Int. J. Health Sci. Res. 5 (2015) 572–584.
[56] V. Adetunji, O. Salawu, Efficacy of ethanolic leaf extracts of Carica papaya and Terminalia catappa as molluscicides against the snail intermediate hosts of schistosomiasis, J. Med. Plants Res. 4 (2010) 2348–2352.
[57] K. Ghimire, J. Banerjee, A.K. Gupta, P. Dahal, Phytochemical constituents and pharmacological uses of medicinal plant Achyranthes aspera: a Review, World J. Pharmaceut. Res. 4 (2015) 470–489.
[58] S. Srivastav, P. Singh, G. Mishra, K. Jha, R. Khosa, Achyranthes aspera-An important medicinal plant: a review, J. Nat. Prod. Plant Resour. 1 (2011) 1–14.
[59] G. Nonviho, V.D. Wotto, J.-P. Noudogbessi, F. Avlessi, M. Akogbeto, D. C. Sohounhloué, Insecticidal activities of essential oils extracted from three species of Poaceae on Anopheles gambiae spp, major vector of malaria, St. Cerc. St. CICBIA 11 (2010) 411–420.
[60] A.J. Aladesanmi, Tetrapleura tetraptera: molluscicidal activity and chemical constituents, Afr. J. Tradit., Complementary Altern. Med. 4 (2007) 23–36.
[61] R. Ojutiku, E. Avbarefe, R. Kolo, F. Asuwaju, Toxicity of Parkia biglobosa pod extract on Clarias gariepinus juveniles, Int. J. Fish. Aquacult. 4 (2012) 133–138.
[62] O. Orji, U. Ibiam, P. Aja, Acute toxicity studies of the lyophilized aqueous extract of Psychotria microphylla leaf on Clarias gariepinus juveniles, Int. J. Bioinf. Biol. Sci. 3 (2014) 38–44.