Analysis of Achyranthes aspera leaf extract and environmental safety evaluation to non target Nile tilapia fish fingerlings, Oreochromis niloticus.

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Research

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Analysis of *Achyranthes aspera* leaf extract and environmental safety evaluation to non target Nile tilapia fish fingerlings, *Oreochromis niloticus*.

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Abstract

Background: Although plant molluscicides are biodegradable and less toxic to higher animals, unregulated applications could affect other non target aquatic species. Eco toxicological studies are required to evaluate whether they are safe to those economically and ecologically important fish species. According to previous studies, Achyranthes aspera is a molluscicidal plant with LC$_{90}$ of 96.5 mg/L and the current study is to investigate its toxic effect to Nile tilapia fish fingerlings, Oreochromis niloticus.

Methods: Seven fish fingerlings were exposed to serial dilutions of A. aspera leaf aqueous extract for 96 hours in duplicate setup. Phytoconstituents were identified by GC-MS and lethal concentrations were determined by probit model. The NOAEC was determined by hypothesis testing on the survival data.

Results: The respective piscicidal LC$_1$, LC$_{10}$ and LC$_{50}$ values were 897.4, 1063.9 and 1310.74 mg/L. The NOAEC was 1100 mg/L (p>0.05). GC-MS analysis revealed 12 phytoconstituents including a monoterpene.

Conclusion: The result shows that A. aspera is non toxic and hence is safe to Nile tilapia fish especially at its molluscicidal dose limit (96.5 mg/L LC$_{90}$) corroborating to the plant’s target specific molluscicidal potential.

Keywords: Achyranthes aspera, molluscicidal, NOAEC, Oreochromis niloticus, piscicidal, phytochemical
Background

Awareness on the detrimental impact of synthetic pesticides on the environment coupled with increasing prices is forcing researchers and communities to look for alternative botanical products [1]. Ease of access at low cost and environmental friendliness are 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[2, 3].

Even though, plant molluscicides are generally considered as ecofriendly, rapidly biodegradable and less toxic to higher animals, their indiscriminate use could cause risk to many nontarget organisms. Toxic effects of these products at least to those important species should be investigated beforehand. Lack of such ecotoxicological information is a hindrance to their practical applicability[4, 5] Acute fish toxicity assessments were performed on few molluscicidal plants. For instance, the toxicity effect of *Phytolacca dodecandra* to aquatic invertebrates *Daphnia Magna* and fish (*Pimephales Promelas*) was discussed in a study by Lambert et al., [6]. Similarly, toxicity of *Jatropha curcas* on certain aquatic crustaceans and annelids was evaluated by Rug and Ruppel [7]. Acute toxicity of *Moringa oleifera* to Nile tilapia *Oreochromis niloticus* fingerlings is also studied [4, 8].

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 [9]. In addition, identification of bioactive compounds is useful for further understanding of its molluscicidal properties and for the synthesis of effective chemical substances [10].
Achyranthes aspera, (Amaranthaceae), also known as “devil's horsewhip” is stiff erect perennial herb growing as a weed in many parts of Asia, Africa, America, Europe and Australia [11–13]. It is a well-known medicinal plant in Ethiopia, India and other countries as antifungal, antibacterial, antioxidant, antifertility and in the treatment of renal dropsy, skin rash, chronic malaria, impotence, asthma, diabetes etc.[14–16]. Very recently, The molluscicidal property of A. aspera is discovered by after investigation on the aqueous and ethanolic extracts as well as in bait formulation [17, 18].

Phytochemical analyses carried out on A. aspera have revealed several compounds including flavonoids, alkaloids, saponins and cardiac glycosides.[16, 19–21]. Saponins and alkaloids are molluscicidal; also, piscicidal. Therefore, the plant’s molluscicidal application requires further phytochemical analysis and ecotoxicological investigations.

This study is aimed to identify the major phytoconstituents present in A. aspera leaves and investigate its acute toxicity to fingerlings of Nile tilapia, O. niloticus.

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 [22–25]. Moreover, it is one of the predominantly stocked fish in aquacultures, artificial lakes, reservoirs and small water bodies.

**Materials and methods**

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

**Plant Material Collection and Processing**

Fresh leaves of A. aspera plant are collected in October 2017 from a natural habitat
located at 9°43'45.59" N, 39°37'2.71" E in Debre Berhan Zuria-Keyit District in Amhara region, Ethiopia. The leaves were dried in the shade to a final weight and ground to a 200 \( \mu \)m mesh sized powder using an electric blender. For each serial dilution, crude aqueous extraction was made at the time of experimentation, by soaking the required amount in 400 ml aged water in a conical flask and shaking overnight in an orbital shaker at 160 rpm. Finally, it was filtered using a clean cotton filtering cloth where the filtrate was then transferred in to a volumetric flask, tightly capped and stored in the refrigerator at 4°C.

**Test Animal Collection and Maintenance**

Fingerlings of *O. niloticus* were collected from an artificial fishpond in Dilla University main compass, southern Ethiopia. The owner of the pond, Department of Biology, has permitted us to take 110 fingerlings. A skilled person assigned to manage the pond, caught the fish with care by a small handmade fishing net. Both male and female fingerlings of length 5.9-6.6 cm (6.27±0.2 cm) and mass 3.4-4.3 g (3.89±0.25 g) were selected for this test and the remaining were immediately returned in to the pond. Caught fish were promptly put into clean plastic buckets containing the pond water and immediately brought to the laboratory for acclimatization. They were maintained in two 80 cm x 60 cm x 60 cm aquaria for one week in aged tap water under continuous aeration and 12-hour light, 12-hour dark photoperiod. They were fed with flakes of tasty soya (Pramukh Agroindustry PLC, Ethiopia) as recommended [26]. Feeding was stopped 24 hours prior to the start of experiment [27, 28].

**Range Finding Test**

The range finding test was set according to the protocols defined by OECD [29] and EPA [30]. 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 hours. The purpose of the range finding test is to identify the useful
collection range that would probably produce mortality rates between 0 and 100%
and guess the range of concentrations for the definitive test.

**Definitive Test**

Six serial dilutions: 600, 800, 1000, 1100, 1200 and 1400 mg/L were prepared in 30 L
aged tap water. According to the protocols [29, 31], seven healthy fishes were randomly
released in to each test solution for 96-hour exposure time. Only aged tap water was used
for negative control. The whole test was prepared in duplicates. Each solution and the
controls were intermittently aerated for about 40 minutes in every 2-3 hours.

**Physicochemical Characteristics of Test Water**

Dissolved oxygen, temperature and pH were measured in every 24 hours using a
multiparameter probe. Before every measurement, the probe was calibrated according to
its operating manual. Total hardness was measured only once at the start by EDTA
titrimetric method. Eriochrome Black T (EBT) was used as indicator. First, 100 ml sample
water was buffered by 2 ml ammonium hydroxide to pH 10. When few drops of EBT was
added to this sample water, a wine-red color appeared. Upon titration with EDTA, the
color gradually changes to blue. At this instance of color change, the titration was
Immediately stopped and the final volume of the ample water was noted

\[
\text{Hardness (in mg/L as CaCO}_3\text{) } = \frac{(V \times N \times 50 \times 1000)}{SV}
\]

Where: \(V\) = volume of titrant (ml); \(N\) = normality of EDTA; \(50\) = equivalent weight of
\(\text{CaCO}_3\); \(SV\) = sample volume (ml)
The course of the experiment was monitored through constant and careful bench side observation and cumulative mortalities were recorded at the end of 12, 24, 48, 72 and 96 hours according to OECD [29]. Dead fish were identified by absence of tail and gill movements and loss of sensation to gentle prodding with a glass rod [32]. Dead fish were immediately picked out from the solutions to minimize contamination.

**Phytochemical Analysis of A. aspera by GC-MS**

The chemical profile of *A. aspera* leaf crude ethanolic extract was documented from GC-MS analysis employing Agilent-Technologies (Little Falls, CA, USA) 6890N Network Gas Chromatographic system, equipped with an inert XL Mass detector (Agilent-Technologies 5975), auto injector (Agilent-Technologies 7683B series) and HP-5MS 5% Phenyl Methyl Silox capillary column (27 m x 250 μm with film thickness x 0.24 μm). The cured extract was clean up by silica gel and dissolved in HPLC grade n-hexane with a ratio of 1:99. This mix was ready for GC-MS analysis. 1.0 μL sample was injected in the split less mode. Helium was used as a carrier gas with a flow rate of 1 ml/min. The temperature of column oven was programmed started from 40 °C for 0 min to 100 °C at 4 °C/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. An electron ionization system (with ionization energy of 70eV) was used for GC/MS detection while the scanning mass was ranged from 33–500 m/z.

**Data Analysis**

The phytoconstituents were identified by comparing the spectra of each unknown component with the spectrum 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, version 23. Mean and standard deviations of fish weight and lengths as well as the water physicochemical parameters were computed in Microsoft excel spreadsheet of Office 2016.

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 [30, 33]. In other words, it is the highest concentration from the serial dilutions in which the number of fishes died is not significantly different from that in the control. Therefore, the NOAEC was determined by hypothesis testing using Dunnett t test at 95% confidence interval [30].

First: the mortality rates are recorded as response proportion data for each concentration and control group.

\[ (RP) = \frac{Number \, of \, survivors \, / \, Total \, exposed; \, where \, RP \, is \, Response \, Proportion }{ } \]

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

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

\[ Angle \, (in \, radians) = \frac{arc \, sine}{\sqrt{(RP)}} \]

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

\[ Angle \, (in \, radians) = \frac{arc \, sine}{\sqrt{1/4n}} \]

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 transformation of the corresponding mortality data at 95% confidence interval to determine which maximum concentration exhibited statistically insignificant difference from the control.

**Results**

*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 hours. 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.

**Physicochemical Properties of the Test Water**

Lower level of dissolved oxygen was detected in all test solutions other than the control in every measurement. On the other hand, pH measurements of the first day were higher than those in the other days. Standard deviation of the temperature recorded was less than the 1.5 maximum limit set by OECD for a valid test [29]. In addition, all dissolved oxygen measurements were above 60%.

**Range Finding Test Results**

In the range finding test, the 100, 400 and 800 mg/L dilutions did not kill any fish. Rather, fish death was observed at 1200 and 1600 mg/L solutions where, the former killed two
and the later killed all the three. Based on the result, five equidistant concentrations between 800 and 1600 mg/L were suggested for the definitive test. The researchers discussed on it and decided to include a 600 mg/L dilution and avoided the upper most 1600 mg/L level. The underlying rationale was that, as the exposure time extends from 24 to 96 hours, fish would become weaker due to prolonged starvation and hence, their resistance declines. On the other hand, prolonged exposure could aggravate toxic effects. Thus, serial dilutions of 600, 800, 1000, 1100, 1200 and 1400 mg/L were set for the definitive test.

**Definitive Test Results**

The definitive test was started with the expectation that, the extended exposure time (96 hours) will increase toxicity of the extract. As a result, fish mortality will be higher in the third and fourth day than in the first and second. However, contrary to this, the mortality data shows that most fish deaths were recorded in the first 48 hours (Table 1).

<table>
<thead>
<tr>
<th>Test conc. (mg/L)</th>
<th>Number of fish exposed</th>
<th>Fish cumulative mortalities at different time intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 hours</td>
</tr>
<tr>
<td>0 (Control)</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>600</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>800</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>1000</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>1100</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>1200</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>1400</td>
<td>14</td>
<td>3</td>
</tr>
</tbody>
</table>

When the test solutions were discarded at the end, a large amount of fish faeces is found decanted at the bottom of the containers but only in the experimental groups. No fish has
died in the control as well as in the lower 600 and 800 mg/L dilutions nor did the highest 1400 mg/L concentration kill all fishes. Dying fish gradually lose activity and become sluggish, unable to escape when touched. They continuously swirl about unguided and finally sank down.

Table 2. Lethal concentrations and confidence intervals resulted from probit analysis

<table>
<thead>
<tr>
<th>Lethal concentrations</th>
<th>Concentration in (mg/L)</th>
<th>Confidence interval (at 95%)</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC1</td>
<td>897.43</td>
<td>642.48-1006.01</td>
<td>1.02</td>
</tr>
<tr>
<td>LC10</td>
<td>1063.87</td>
<td>903.01-1140.89</td>
<td></td>
</tr>
<tr>
<td>LC50</td>
<td>1310.74</td>
<td>1229.53-1484.42</td>
<td></td>
</tr>
</tbody>
</table>

Concentrations that can cause 1% and 10% mortalities as well as median lethal concentration with corresponding upper and lower limits, in 96-hour exposure were determined from the mortality data by probit analysis (Table 2). Within the 95% confidence interval, the calculated χ² is less than the corresponding tabular value asserting the model’s goodness-of-fit.

The response proportions (RP), or survival proportions and the corresponding arc sine square root transformed values in each test solution of the two replicas are summarized in table 3. The results of Dunnett test on the arc sine square root transformed RP data showed that the highest concentration in which survival of the fingerlings is not significantly different from the control is the 1100 mg/L solution (p>0.05). This level is therefore, the NOAEC.

Table 3. Arc sine transformation of survival proportion data

| Replicate | RP in each test concentration and the control |
Phytochemical Analysis of *A. aspera* Leaf Ethanolic Extract

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

Discussion

*Achyranthes aspera* is a well-known medicinal plant traditionally used for ailments of various diseases in many parts of the world [14]. In addition, the molluscicidal potential of this plant is recently discovered [17] 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.

Regarding the toxicity effect of different molluscicidal plants on fishes, *Sapindus*
mukorossi exhibited an LC$_{50}$ of 10 ppm while its molluscicidal LC$_{50}$ is 119.57 ppm [34]. *Jatropha gossypifolia* had piscicidal LC$_{50}$ of 10.490 mg/L [35] while its molluscicidal LC$_{50}$ is over 100 ppm [36][36]. The piscicidal LC$_{50}$ of Endod (*Phytolacca dodecandra*) is 4.4 mg/L and its molluscicidal LC$_{50}$ is 10 ppm [37, 6]. 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 [38]. These data indicate the molluscicidal median lethal dose (LC$_{50}$) of each plant exceeds its respective piscicidal LC$_{50}$. And hence, they are more toxic to those nontarget fishes 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. Similarly, 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.,[18]. The considerable gap between the molluscicidal and piscicidal lethal doses of *A. aspera* indicates that the plant has very low or negligible toxic effect on this economically and ecologically valuable fish, *O. niloticus*, especially at its molluscicidal dose limits.

Several phytochemical analyses of the plant have been carried out by different researchers, and they were able to identify different classes of saponins [11, 39, 40]. 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 [41]. The molluscicidal effect of terpenoids is indicated in many literature [4, 42]. In addition, the larvicidal property of eicosanoic acids, and phthalates is indicated in many literatures. In
general, bioactivity of crude extracts usually arises from synergistic effect of several constituents.

In all measurement instances of the test water physico chemical parameters, lower dissolved oxygen level was recorded in test solutions than in the control. This phenomenon agrees with the studies of Ayuba et al.,[32] and Ojutiku et al., [43]. Biodegradation or decomposition of the phytochemicals leads to higher biological and chemical oxygen demand (BOD, COD) and causes depletion of oxygen in the solutions [30, 43].

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 [27, 35, 44]. The reactions could be due to dissolved oxygen depletion by the chemicals leading to oxidative stress [43]. It can also be a manifestation of neuro toxicity and poisoning of the gills by the toxicants [35, 44].

**Conclusion**

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

Molluscicidal plants application in the field can be promoted when molluscicidal efficacy studies are supported by eco toxicological safety data. This particular study showed that *A. aspera* aqueous extract is almost nontoxic to *O. niloticus* fingerlings and is safe to this
fish when applied under its molluscicidal dose limit which is below 100ppm. Therefore, it is possible to conclude that application of the plant product for snail control at molluscicidal effective concentrations of below 100 ppm does not acutely toxify this fish species. However, further studies involving additional non-target groups such as aquatic invertebrates and mammals should be conducted to generate a more complete ecotoxicological information.

**Acknowledgements**

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**Availability of data and materials**

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

**Authors’ contributions**

BM: Contributed in the conception of the study, carried out the experiment,
and wrote the manuscript. STM: Modified the experimental design, supervised the experiment process, and edited the manuscript. AA: Carried out the statistical analysis, organized literatures and edited the final version of the manuscript. YT: Did the phytochemical analysis section and edited the revised manuscript. WM: Carried out water physicochemical measurements and edited the manuscript. DF: Did the phytochemical analysis using GC-MS. All authors read and approved the final manuscript.

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

Competing interests

All authors of this revised manuscript declare that they have no competing interest regarding this research

References


4. Rocha-filho CAA, Albuquerque LP, Silva LRS, Silva PCB, Coelho LCBB, Navarro


30. EPA. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms 5th ed. EPA. 2002;84; doi:10.1590/S0001-37652012005000059


36. Pereira-Filho AA, France CRC, Oliveira DS, Mendes RJA, Goncalves RJS, Rosa IG. Evaluation of the molluscicidal potential of hydroalcoholic extracts of Jatropha


Tables and Figures

Figure 1. Phytoconstituents of A. aspera leaf ethanolic extract analysed by GC-MS.

(a) Cyclohexane, 1-methyi-4-(2-hydroxyethyl) C9H18O; (b) Benzene, (1-methylpropyl) C10H14; (c) Cis-p-mentha-1(7),8-dien-2-ol C16H16O; (d) 1,2-Benzenedicarboxilic acid, butyl 8-methylnonyl ester C22H34O4; (e) Octadecanoic acid, 2-hydroxy-1,3-propanediylester C39H76O5; (f) Oleic acid,eicosyl eter C38H74O2; (g) Benzene,1,3-diethyl
C₁₀H₁₄; (h) Naphthalene,2-methyl C₁₁H₁₀; (i) Naphthalene,1,7-dimethyl C₁₂H₁₂;
(j) Eicosanoic acid C₂₀H₄₀O₂; (k) Benzene, 1,2,3-trimethyl C₉H₁₂; (l) Oleic acid
C₁₈H₃₄O₂.
Figure 1

Phytoconstituents of A. aspera leaf ethanolic extract analysed by GC-MS. (a) Cyclohexane, 1-methyi-4-(2-hydroxyethyl) C9H18O; (b) Benzene, (1-methylpropyl) C10H14; (c) Cis-p-mentha-1(7),8-dien-2-ol C10H16O; (d) 1,2-Benzenedicarboxilic acid, butyl 8-methyleneonyl ester C22H34O4; (e) Octadecanoic acid, 2-hydroxy-
1,3-propanediylester C₃₉H₇₆O₅; (f) Oleic acid, eicosyl eter C₃₈H₇₄O₂; (g) Benzene, 1,3-diethyl C₁₀H₁₄; (h) Naphthalene, 2-methyl C₁₁H₁₀; (i) Naphthalene, 1,7-dimethyl C₁₂H₁₂; (j) Eicosanoic acid C₂₀H₄₀O₂; (k) Benzene, 1,2,3-trimethyl C₉H₁₂; (l) Oleic acid C₁₈H₃₄O₂.