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

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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

94 located at 9°43'45.59" N, 39°37'2.71" E in Debre Berhan Zuria-Keyit District in Amhara
95 region, Ethiopia. The leaves were dried in the shade to a final weight and ground to a 200
96 µm mesh sized powder using an electric blender. For each serial dilution, crude aqueous
97 extraction was made at the time of experimentation, by soaking the required amount in
98 400 ml aged water in a conical flask and shaking overnight in an orbital shaker at 160
99 rpm. Finally, it was filtered using a clean cotton filtering cloth where the filtrate was then
100 transferred in to a volumetric flask, tightly capped and stored in the refrigerator at 4°C.

101 ***Test Animal Collection and Maintenance***

102 Fingerlings of *O. niloticus* were collected from an artificial fishpond in Dilla University
103 main compass, southern Ethiopia. The owner of the pond, Department of Biology, has
104 permitted us to take 110 fingerlings. A skilled person assigned to manage the pond, caught
105 the fish with care by a small handmade fishing net. Both male and female fingerlings of
106 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
107 test and the remaining were immediately returned in to the pond. Caught fish were
108 promptly put into clean plastic buckets containing the pond water and immediately
109 brought to the laboratory for acclimatization. They were maintained in two 80 cm x 60
110 cm x 60 cm aquaria for one week in aged tap water under continuous aeration and 12-
111 hour light, 12-hour dark photoperiod. They were fed with flakes of tasty soya (Pramukh
112 Agroindustry PLC, Ethiopia) as recommended [26]. Feeding was stopped 24 hours prior
113 to the start of experiment [27, 28].

114 ***Range Finding Test***

115 The range finding test was set according to the protocols defined by OECD [29] and EPA
116 [30]. Five serial dilutions of 100, 400, 800, 1200 and 1600 mg/L aqueous extract were
117 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 concentration 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

$$Hardness\ (in\ mg/L\ as\ CaCO3)\ =\ (V \times N \times 50 \times 1000)\ /\ (SV)$$

Where: V = volume of titrant (ml); N = normality of EDTA; 50 = equivalent weight of $CaCO_3$; 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 ^{0}C for 0 min to 100 ^{0}C at 4 ^{0}C /min and from 100 to 310 ^{0}C at 10 ^{0}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 ^{0}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.

$(RP) = Number\ of\ survivors\ /\ Total\ exposed$; 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

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

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

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

where n = number of animals per treatment replica.

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

$$Angle = 1.5708\, radians - (radians\ for\ RP = 0)$$

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

Test conc. (mg/L)	Number of fish exposed	Fish cumulative mortalities at different time intervals			
		24 hours	48 hours	72 hours	96 hours
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

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 loose 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

Lethal concentrations	Concentration in (mg/L)	Confidence interval (at 95%)	$\chi 2$
LC1	897.43	642.48-1006.01	1.02
LC10	1063.87	903.01-1140.89	
LC50	1310.74	1229.53-1484.42	

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 χ^2 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

		Control	600 mg/L	800 mg/L	1000 mg/L	1100 mg/L	1200 mg/L	1400 mg/L
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

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 619.1to 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

283 general, bioactivity of crude extracts usually arises from synergistic effect of several
284 constituents.

285 In all measurement instances of the test water physico chemical parameters, lower
286 dissolved oxygen level was recorded in test solutions than in the control. This
287 phenomenon agrees with the studies of Ayuba et al.,[32] and Ojutiku et al., [43].
288 Biodegradation or decomposition of the phytochemicals leads to higher biological and
289 chemical oxygen demand (BOD, COD) and causes depletion of oxygen in the solutions
290 [30, 43].

291 Fingerlings in the test solution were seen motionless resting at the bottom or incessantly
292 gulping at the water air interface. These behavioural changes also happened in many
293 similar studies [27, 35, 44]. The reactions could be due to dissolved oxygen depletion by
294 the chemicals leading to oxidative stress [43]. It can also be a manifestation of neuro
295 toxicity and poisoning of the gills by the toxicants [35, 44].

296 **Conclusion**

297 As a general principle, molluscicides are directly applied in to the snail infested water
298 body where many other non-target and valuable species co-exist. As a result, they are
299 always subjected to deleterious toxicity by such chemicals applied in to their common
300 habitats. To mitigate such ecological damage, the molluscicide should be selective in
301 action or the dose limit applied for snail control is proved to be safe to such non-target
302 species.

303 Molluscicidal plants application in the field can be promoted when molluscicidal efficacy
304 studies are supported by eco toxicological safety data. This particular study showed that
305 *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.

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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

1. Medhi SM, Reza S, Mahnaz K, Reza AM, Abbas H, Fatemeh M, et al. Phytochemistry and larvicidal activity of *Eucalyptus camaldulensis* against malaria vector , *Anopheles stephensi*. Asian Paci J Trop Med. 2010;841–5; doi:10.1016/S1995-7645(10)60203-9.

2. Otarigho B, Morenikeji OA. Molluscicidal effects of aqueous and ethanolic extracts of Lemongrass (*Cymbopogon citratus*) leaf against the different developmental stages of *Biomphalaria pfeifferi*. NY Sci J. 2012;5:70–7.

3. Forstinus NO, Ikechukwu NE, Emenike MP, Osita O. Synthetic insecticides, phytochemicals and mosquito resistance. Acad J Biotechnol. 2017;5:118–25.

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

355 DMAF, et al. Assessment of toxicity of *Moringa oleifera* flower extract to *Biomphalaria*
356 *glabrata* , *Schistosoma mansoni* and *Artemia salina*. Chemosphere. 2015;132:188–92;
357 doi: 10.1016/j.chemosphere.2015.03.041.

358 5. Singh A, Singh D, Misra T, Agarwal R. Molluscicides of plant origin,biological
359 agriculture & horticulture. Int J Sustain Prod Syst. 2012;13:205–52;
360 doi:10.1080/01448765.1996.9754782

361 6. Lambert J, Temmink J, Marquis J, Parkhurst R, Lugt C, Lemmich E, et al. Endod:
362 Safety evaluation of a plant molluscicide. Regul Toxicol Pharmacol. 1991;14:189-201.

363 7. Rug M, Ruppel A. Toxic activities of the plant *Jatropha curcas* against intermediate
364 snail hosts and larvae of schistosomes. Trop Med Intern Heal. 2000;5:423–430.

365 8. Ayotunde EO, Fagbenro OA, Adebayo OT. Toxicity of aqueous extract of *Moringa*
366 *oleifera* seed powder to Nile tilapia *Oreochromis niloticus* (LINNE I779), fingerlings. Int
367 Res J Agric Sci Soil Sci. 2011;1:142–50.

368 9. Vivekraj P, Vinotha S, Vijayan A, Anand G V. Preliminary phytochemical screening
369 and GC-MS analysis of methanolic extract of *Turnera subulata* Smith (Passifloraceae).
370 J Phytopharm. 2017;6:174–7.

371 10. Nandagopalan V, Gritto MJ, Doss A. GC-MS analysis of bioactive components of the
372 methanol extract of *Hibiscus tiliaceus* Linn . Asian J Plant Sci Res. 2015;5:6–10.

373 11. Sharma A, Kumar S, Tripathi P. Impact of *Achyranthes aspera* leaf and stem extracts
374 on the survival , morphology and behaviour of an Indian strain of dengue vector , *Aedes*
375 *aegypti* L . (Diptera : Culicidae). J Mosq Res. 2017;5:1–9;
376 doi:10.5376/jmr.2015.05.0007

377 12. Sharma V, Chaudhary U, Singh R, Agarwal A. *Achyranthes aspera*: phytochemical
378 estimation. Am J PharmTech Res. 2013;3:242–51.

379 13. Eshete MA, Asfaw Z, Kelbessa E. A review on taxonomic and use diversity of the
380 family Amaranthaceae in Ethiopia. J Med Plants Stud. 2016;4:185–94.

381 14. Habtamu A, Mekonnen Y. Evaluation of the antibacterial activities of leaf extracts of
382 *Achyranthus aspera*. Afr J Bacteriol Res. 2017;9:9–14; doi:10.5897/JBR2015.0189

15. Reddy CV, Kamble A. Toxicity study of *Achyranthus aspera*. Int Lett Nat Sci. 2014;14:85–96; doi: 10.18052/www.scipress.com/ILNS.14.85

16. Reddy RBD, Reddy PN, Prathibha N, Mounica TM, Phanikumar A, Ravindra M. Pharmacognostic, preliminary phyto chemical and pharmacological studies on the roots of *Achyranthes aspera*. IJPPR. 2012;4:139–41.

17. Mandefro B, Mereta ST, Ambelu A. Efficacy of *Achyranthes aspera* (L .) as a molluscicidal bait formulation against fresh water snail *Biomphalaria pfeifferi*. Evidence-Based Complement Altern Med. 2018;2018:1–7; doi:10.1155/2018/2718585

18. Mandefro B, Mereta ST, Tariku Y, Ambelu A. Molluscicidal effect of *Achyranthes aspera* L. (Amaranthaceae) aqueous extract on adult snails of *Biomphalaria pfeifferi* and *Lymnaea natalensis*. Infect Dis Poverty. 2017;6; doi:10.1186/s40249-017-0349-4

19. Dey A. *Achyranthes aspera* L: phytochemical and pharmacological aspects. Int J Pharm Sci Rev Res. 2011;9.

20. Varadharaj V, Kuppan M. Identification and determination of bioactive phytochemical constituents from the hydro-alcoholic extract of *Achyranthes aspera* whole plant by gas chromatography-mass spectrometry analysis. 2015;8:6–10.

21. Kumar V, Sharma A, Thukral AK, Bhardwaj R. Phytochemical profiling of methanolic extracts of medicinal plants using GC-MS. Int J Res Dev Pharm L Sci. 2016;5:2153–8.

22. Breuil C, Damien G. Fisheries in the ESA-IO Region: Profile and trends Country review 2014. Ebene; 2014.

23. Endebu M, Lema A, Genet T, Mitike A, Regassa B, Dejen E, et al. Fisheries baseline survey describing status of fisheries in Lake Zeway, Ethiopia. J Fish Livest Prod. 2015;3; doi:10.4172/2332-2608.1000129

24. Janko AM. Economics and management sciences fish production , Consumption and management in Ethiopia. Int J Econ Manag. 2014;3; doi:10.4172/2162-6359.1000183

25. Tesfahun A. Review in current problems of Ethiopian fishery: Incase of human and natural associated impacts on water bodies. Int J Fish Aquat Stud. 2018;6:94–9.

411 26. Kushwaha MP. Replacement of fish meal by soybean (Glycine max) in the
412 formulation of fish feed ingredients essential for immunostimulation and growth
413 performance of carps. Int J Fauna Biol Stud. 2013;1:35–8.

414 27. Okey I, Keremah R, Ikpi G, Ebeniro L. Toxicity of leaf powder of *Lepidagathis*
415 *alopecuroides* to Nile tilapia *Oreochromis niloticus* juveniles. Afr J Food Sci.
416 2013;7:128–33; doi:10.5897/AJFS11.126

417 28. Syngai G, Dey S, Bharali R. Evaluation of toxicity levels of the aqueous extract of
418 *Allium sativum* and its effects on the behavior of juvenile common carp (*Cyprinus carpio*
419 l., 1758). Asian J Pharm Clin Res. 2016;9:417–21.

420 29. OECD. Guideline for Testing of Chemicals: Fish , Acute Toxicity Test (Draft revised
421 version). 2014.

422 30. EPA. Methods for measuring the acute toxicity of effluents and receiving waters to
423 freshwater and marine organisms 5th ed. EPA. 2002;84; doi:10.1590/S0001-
424 37652012005000059

425 31. EPA. Ecological effects test guidelines fish acute toxicity test , freshwater and marine.
426 1996. Washington, DC.

427 32. Ayuba VO, Ofojekwu PC, Musa SO. Acute toxicity of *Clarias gariepinus* exposed to
428 *Datura innoxia* leaf extract. J Med Plants Res. 2012;6:2453–7;
429 doi:10.5897/JMPR11.1641

430 33. Pereyra PJ, Rossini GP, Darrigran G. Toxicity of neem's oil, a potential biocide
431 against the invasive mussel *Limnoperna fortunei* (Dunker 1857). An Acad Bras Ciênc.
432 2012;84; doi:10.1590/S0001-37652012005000059.

433 34. Upadhyay A, Singh DK. Pharmacological effects of *Sapindus mukorossi*. Rev Inst
434 Med Trop Sao Paulo. 2012;54:273–80; doi:10.1590/S0036-46652012000500007

435 35. Singh P, Singh A. Acute toxic effects of medicinal plant *Jatropha gossypifolia* on
436 non- target fish and mice. Wudpecker J Agric Res. 2012;1:433–8.

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

gossypiifolia Linnaeus, 1753 on *Biomphalaria glabrata* (Say, 1818). Rev Inst Med Trop Sao Paulo. 2014;56:505–10; 10.1590/S0036-46652014000600009

37. Belete EM. Schistosomiasis Control Strategies, with Emphasis on Snail Control Using Molluscicides. Int J Heal Sci Res. 2015;5:572–84.

38. Adetunji VO, Salawu OT. Efficacy of ethanolic leaf extracts of *Carica papaya* and *Terminalia catappa* as molluscicides against the snail intermediate hosts of schistosomiasis. J Med Plants Res. 2010;4:2348–52; doi:10.5897/JMPR10.468.

39. Ghimire K, Banerjee J, Gupta AK, Dahal P. Phytochemical constituents and pharmacological uses of medicinal plant *Achyranthes aspera*: a review. World J Pharm Res. 2015;4:470–89.

40. Srivastav S, Singh P, Mishra G, Jha K, Khosa R. *Achyranthes aspera*-An important medicinal plant: A review. J Nat Prod Plant Resour. 2011;1:1–14.

41. Nonviho G, Wotto VD, Noudogbessi J, Avlessi F, Akogbeto M, Sohounhloué DCK. Insecticidal activities of essential oils extracted from three species of Poaceae on *Anopheles gambiae* spp , major vector of malaria. St Cerc St CICBIA. 2010;11:411–20.

42. Aladesanmi, A J. *Tetrapleura tetraptera*: Molluscicidal activity and chemical constituents. Afr J Trad CAM. 2007;4:23–36.

43. Ojutiku R, Avbarefe E, Kolo R, Asuwaju F. Toxicity of *Parkia biglobosa* pod extract on *Clarias gariepinus* juveniles. Int J Fish Aquac. 2012;4:133–8; doi:10.5897/IJFA11.118

44. Orji OU, Ibiam UA, Aja PM. Acute toxicity studies of the lyophilized aqueous extract of *Psychotria microphylla* leaf on *Clarias gariepinus* juveniles. Int J Biol Biol Sci. 2014;3 June:38–44.

Tables and Figures

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

(a) Cyclohexane, 1-methyi-4-(2-hydroxyethyl) $C_9H_{18}O$; (b) Benzene, (1-methylpropyl) $C_{10}H_{14}$; (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 $C_{18}H_{34}O_2$.

Figures

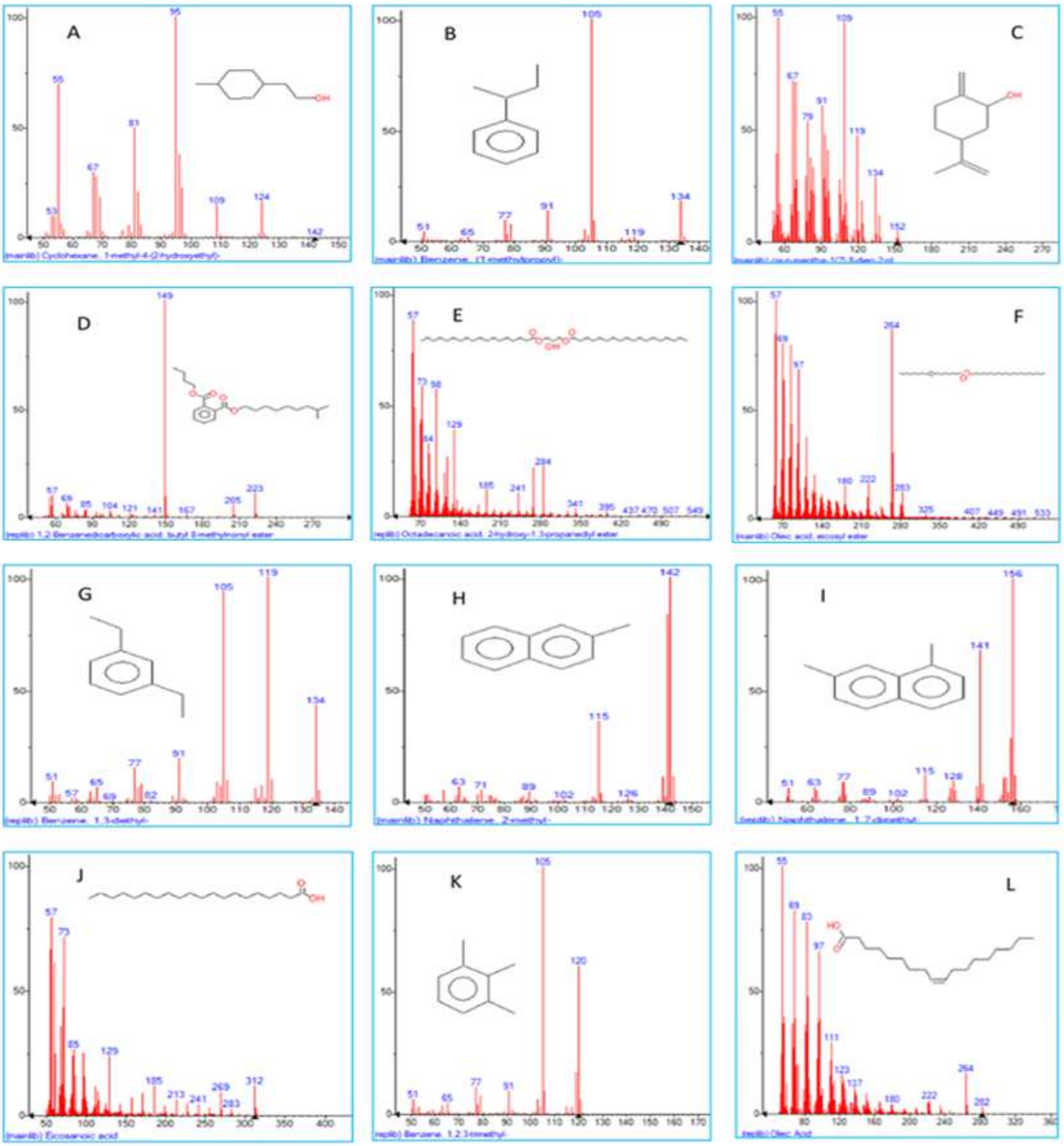

Figure 1

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 C10H16O; (d) 1,2-Benzenedicarboxilic acid, butyl 8-methylnonyl ester C22H34O4; (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 $C_{18}H_{34}O_2$.