European Review for Medical and Pharmacological Sciences 2008; 12: 381-386

Laboratory evaluation of Ethiopian local plant *Phytolacca dodecandra* extract for its toxicity effectiveness against aquatic macroinvertebrates

K. KARUNAMOORTHI, D. BISHAW, T. MULAT

School of Environmental Health Science, Faculty of Public Health, Jimma University, Jimma (Ethiopia)

Abstract. – In this study, we evaluated the toxicity effectiveness of berries crude extract of Endod [vernacular name (local native language, Amharic); *Phytolacca dodecandra*] against aquatic macroinvertebrates Baetidae (Mayflies) and Hydropsychidae (Caddisflies), under laboratory conditions. In Ethiopia, toxic plant, berries of *Phytolacca dodecandra* are being commonly used for washing clothes and to control fresh water snails. Macroinvertebrates are useful biological indicators of change in the aquatic ecosystems. The present study clearly revealed that the LC_{50} and LC_{90} values for berries crude extract of *Phytolacca dodecandra* against Baetidae were 181.94 and 525.78 mg/l and lethal doses (LC_{50} and LC_{90}) required for Hydropsychidae were 1060.69 and 4120.4 mg/l respectively. The present investigation demonstrated that Baetidae was more susceptible than Hydropsychidae, even at shorter exposure period of 2 h. From our preliminary investigation the toxicity effectiveness of crude extracts of *Phytolacca dodecandra* has been clearly shown. In addition, it requires further explorations which address both the toxicity activity and the active principles that are responsible for its toxicity effectiveness. Ultimately, the release/introduction of *Phytolacca dodecandra* plant berries extracts into the river/streams leads to disruption of food chain in the aquatic ecosystem. Therefore, at this moment preserving the aquatic ecosystem is extremely essential and inevitable.

Key Words:

Plant extracts, *Phytolacca dodecandra*, Baetidae, Hydropsychidae, Ethiopia, Toxicity, Aquatic ecosystem, Macroinvertebrates.

Introduction

The Ethiopian flora is estimated to contain between 6,500 and 7,000 species of higher plants, of which about 12% are endemic[1]. Plants have been used as a source of medicine in Ethiopia from time immemorial to treat different ailments[2]. In Ethiopia, toxic plants, berries of *Phytolacca dodecandra* are being commonly used for washing clothes and to control fresh water snails. Since the discovery of Endod in 1965, there have been extensive studies on the chemistry, toxicity, and epidemiology of Lemmatoxins, together with cultivation of the Endod plant[3]. Kloos and McCullough (1984)[4] reported that more than 1000 plants have been screened for molluscicidal activities and immature berries of *Phytolacca dodecandra* have been found to have the most potent molluscicides.

The Endod (*Phytolacca dodecandra*)-based schistosomiasis mansoni control project was implemented in Ethiopia between 1994 and 1999. The aim was to develop an effective, cheap and sustainable method of controlling schistosomiasis[5]. Systemic screening selection of some 600 wild types of Endod plants indicated that berries of a *Phytolacca* species, type 44, in Ethiopia contained as much as 25% by weight of saponins, from which the molluscicides, Lemmatoxins, have been isolated and purified with organic solvents[6]. Because of the detergent properties of Endod plants in combination with additives, Endod powder has been used to produce laundry detergents.

Endod is a proven botanical pesticide with LC_{50} of 1.85 ppm to control schistosomiasis transmitting snails. Because of its larvicidal effects it can be used against larva of mosquito and other insects such as the housefly[7]. To date, several studies have been described on biological evaluations of Endod [vernacular name (local native language, Amharic); *Phytolacca dodecandra*] and

Corresponding Author: Kaliyaperumal Karunamoorthi, M.Sc., M.Phil. DPHM; e-mail: k_karunamoorthi@yahoo.com

concentrated around its effects on snails, and their medicinal as well as antifungal properties. Only very limited studies focused towards their toxicity effect on the aquatic ecosystem and their toxicity potential against macroinvertebrates.

The toxicity effects of chemicals on aquatic organisms is studied worldwide by using aquatic toxicity tests in which macroinvertebrates are being used as test animals because they are the good indicators of the life supporting capacity of aquatic ecosystem. Therefore, it is an hour to launch to test their toxicity effectiveness of Endod berries extract (*Phytolacca dodecandra*) against aquatic macroinvertebrates viz, Baetidae and Hydropsychidae.

Objectives

The aim of this present investigation was to evaluate the most commonly known Ethiopian local plant Endod berries extract (*Phytolacca dodecandra*) for their toxicity effectiveness against aquatic macroinvertebrates, under the laboratory conditions.

Materials and Methods

Plant Selection

In this present study a plant with known toxicity properties was selected from secondary data i.e. some reports in the literature or some bioethnological knowledge by the farmers, fisherman and local residents. Plant species Endod (*Phytolacca dodecandra*) showing toxicity activities was selected. In Ethiopia, since the prehistoric era the local rural inhabitants have been using this plant for various purposes including medicines.

Endod Plant Details

Toxonomic Position

Phytolacca dodecandra L'Hér.
Family: Phytolaccaceae
Genus: Phytolacca
Species: dodecandra
Other Name: The African soapberry

Endod Discovery

For thousands of years in Ethiopia, the berries of Endod plants have been used as laundry soap (hence the name soapberry) in streams that constitute the major source of drinking water for humans as well as for other animals. These streams also harbor the source of schistosoma that depend on snails as their immediate host prior to infection of some 300 million people in Africa[8]. During an epidemiology survey of schistosomiasis in Northern Ethiopia, Lemma[8] noticed that many snails died downstream from where laundry was done. Subsequent laboratory[9] and field investigations[10] revealed that Endod possessed molluscicidal compounds that are saponins found in almost all green plants. These saponin called Lemmatoxins[11,12].

Physical Characteristics

Endod is a perennial plant. It is a climbing plant with hanging branches growing up to 10 m. Usually, in a year twice it used to provide fruits from December – February and then June – July. Often, it is found in highlands of Ethiopia from 1600-3000 m above the sea level.

General Uses

Parts of the Endod plant have been used as a detergent and as traditional medicine for centuries in Ethiopia. Endod is known as traditional soap in rural Ethiopia and rids clothes of parasites such as lice. It is also used in traditional medicine against dandruff and other skin diseases. People of all ages are familiar with the plant and its detergent and medicinal uses. Endod is considering being associated with poor people. Common medicinal uses include treatment of skin itching (ringworm), abortion, gonorrhea, leeches, intestinal worms, anthrax and rabies[13].

Macroinvertebrates

The term macroinvertebrates is traditionally used to refer to aquatic invertebrates including insects (e.g. larval Ephemeroptera and Trichoptera), crustaceans (e.g. amphipods) and molluscs (e.g. aquatic snails). The most common types of aquatic macroinvertebrates are insects. Macroinvertebrates are an important part of the aquatic food chain. Macroinvertebrates are sensitive to different chemical and physical conditions. Therefore, the richness of macroinvertebrate community composition in a waterbody can be used to provide an estimate of waterbody health. Environmental modifications or pollution can alter macroinvertebrate communities. Macroinvertebrates are sampled in waterbodies

because they are useful biological indicators of change in the aquatic ecosystems. Unlike fish, most aquatic macroinvertebrates cannot move to avoid pollution.

Macroinvertebrates are an important food source for larger animals like fish. Fish, in turn, are a food source for birds, mammals, aquatic snakes, and even humans. The main advantages of using macroinvertebrates is that some have life span of up to a year and greater, they relatively sedentary, have varying sensitivities to changes in water quality and they are easily collected and identified. Particularly, macroinvertebrates like Baetidae (mayflies) and Hydropsychididae (caddisflies) are extremely sensitive to toxicity/pollution. Therefore, in this present study, the toxicity effectiveness of Endod berries extract was evaluated against immature stages of mayflies and caddisflies.

Scientific Classification of Baetidae (Mayflies)

Kingdom	:	Animalia
Phylum	:	Arthropoda
Class	:	Insecta
Order	:	Ephemeroptera
Superfamily	:	Baetoidea
Family	:	Baetidae

Baetidae is a family of mayflies with about 900 described species distributed worldwide. These are among the smallest of mayflies, adults rarely exceeding 10 mm in length excluding the two long slender tails and sometimes much smaller, and members of the family are often referred to as small mayflies or small minnow mayflies. Baetidae breed in a wide range of waters from lakes and streams to ditches and even water butts. The nymphs are strong swimmers and feed mainly on algae[14].

Scientific Classification of Hydropsychididae (Caddisflies)

Kingdom	:	Animalia
Phylum	:	Arthropoda
Class	:	Insecta
Super order	:	Amphiesmenoptera
Order	:	Trichoptera
Family	:	Limnephilidae

Caddis flies have aquatic larvae and are found in a wide variety of habitats such as streams, rivers, lakes, ponds, spring seeps, and temporary waters (vernal pools). The larvae are important food for fish. Almost all caddisfly larvae build cases to live in. Some live in silk cases attached to rocks or logs, some build cases out of stones or sand, and some build theirs out of sticks and grass. Together with stoneflies and mayflies, Caddisflies feature importantly in bioassessment surveys of streams and other water bodies[15].

Macroinvertebrates Collection

Awetu is a river crossing Jimma town in Ethiopia. Sample collection site is nearly 5 km away from the Jimma University main campus. The test organisms both the aquatic macroinvertebrates like Baetidae and Hydropsychidae were collected from the Awetu River near the Jimma University college of Agriculture. During the study period some of the physico-chemical parameters of Awetu River were determined by using the appropriate standard procedures. These parameters were pH, temperature, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), turbidity and conductivity which were 7.25, 20°C, 7.20 mg/l, 1.90 mg/l, 13.50 Formazin Turbidity Unit (FTU) and 160µS (microsiemen) respectively. Test organisms were collected by using macroinvertebrate collection nets, filters and containers. Macroinvertebrates were brought along with river water to the laboratory without any injuries/damage during transport. Later, test organisms were placed in the containers with tap water one day for acclimatization.

Plant Collection and Extraction Preparation

The berries of Endod were collected outskirt of Jimma town, Ethiopia. The berries of Endod were washed with tab water and cut into small pieces. These pieces then shade dried at room temperature (25±2°C) till they were complete dry. Later crushed and powdered with mortal pistil grinder. Later the powders were dissolved in distilled water at the different rates (g/100 ml). The solutions were allowed to stand for 24 hours for extraction. After 24 h the mixture was filtered with cheese cloth and filter paper[16].

Laboratory Evaluation for Toxicity Effectiveness

The toxicity effectiveness of plant extracts against aquatic macroinvertebrates like Baetidae and Hydropsychida*e* were determined by using standard procedure. Testing berries extracts of Endod (*Phytolacca dodecandra*) for toxicity effectiveness was carried out at different concen-

trations ranging from 125 to 2000 ppm. The desired concentrations of test solution were achieved by adding 1 ml of an appropriate stock solution to 249 ml of tap water taken in 500 ml beaker. Twenty five numbers of Baetidae and Hydropsychidae were exposed to various concentrations of plants extracts. Three replicates were run for each concentration and species of Baetidae and Hydropsychidae and simultaneous control with same tap water were set up. The experiments were conducted at 28±2°C and 70-80% relative humidity.

Since species of Baetidae was extremely susceptible to crude extracts of Endod the mortality was recorded 2 h of post treatment. However, on the other hand the mortality of Hydropsychidae was recorded 8 h of post treatment. In recording the percentage effect for each concentration, moribund (about to die) and dead test organisms were considered as affected. LC_{50} value indicated the fifty percent mortality and LC_{90} value indicated the ninety percent mortality were calculated from a series of "exposure" concentration by comparing the percentage of mortality in the treated group to control by probit analysis[17]. Statistical analyses were performed with the SPSS 10.1computer program (SPSS Inc. Chicago, Illinois, USA). The number of macro invertebrates in both control and test were recorded after 2/8 h of post treatment. When the control mortality was between 5-20 percent effects were corrected by using Abbott's formula[18].

$$\text{Corrected mortality} = \frac{T - C}{100 - C} \times 100$$

where,
T = % observed mortality
C = % control mortality

Results

Laboratory evaluation of *Phytolacca dodecandra* extract for toxicity effectiveness against aquatic macroinvertebrates

The toxicity effectiveness of berries crude extract of *Phytolacca dodecandra* was tested against *Baetidae* and *Hydropsychidae* at different concentrations viz., 125, 250, 500 1000 & 2000 ppm and various parameters for the said effects are presented below in the Table I, Table II and Figure 1. Since species of Baetidae extremely susceptible to crude extract of Endod the mortality was recorded 2 h of post treatment. However, on the other hand the mortality of Hydropsychidae was recorded 8 h of post treatment.

The LC_{50} and LC_{90} values for berries crude extract of *Phytolacca dodecandra* against Baetidae were 181.94 mg/l and 525.78 mg/l respectively for 2h. From these values as well as from Figure 1 and Table I it can be seen that Baetidae is more susceptible. The value of χ^2 was 2.98. Similarly, Figure 1 and Table II reveals that the *Phytolacca dodecandra* berries crude extract lethal doses (LC_{50} and LC_{90}) required for Hydropsychidae were 1060.69 mg/l and 4120.4 mg/l respectively for 8 h. The value of χ^2 was 1.72.

Discussion

The present study clearly revealed that the LC_{50} and LC_{90} values for berries crude extract of *Phytolacca dodecandra* against Baetidae were 181.94 mg/l and 525.78 mg/l respectively for 2h. From these values as well as from Figure 1 and Table I it can be seen that Baetidae is more susceptible. Similarly, Figure 1 and Table II reveal that the berries crude extract of *Phytolacca dodecandra* lethal doses (LC_{50} and LC_{90}) required for Hydropsychidae were 1060.69 mg/l and 4120.4 mg/l respectively for 8 h.

The study was conducted to evaluate the toxicity of the butanol extract of Endod (*Phytolacca dodecandra*) on 4 species of aquatic animals. Groups of 10 mosquito fish (*Gambusia affinis*) and 8 bluegill (*Lepomis macrochirus*) were exposed to the butanol extract of Endod in 300 ml of water at concentrations of 0.0, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2 or 2.0 ppm. Groups of 10 tropical snails (*Biomphalaria glabrata*) and 10 pond snails (Physa spp) were also exposed to the crude extract in 50 ml of water at concentrations of 0.0, 1.0, 1.5, 2.0, 2.2, 2.5, 3.0, 4.0 or 5.0 ppm. The butanol extract of Endod was lethal to 50% of the fish and snails at relatively low concentrations (less than 3.0 ppm). The results also indicated that fish were approximately 2 to 4 times more sensitive to Endod than snails[19].

There was a similar study was investigated by testing different formulations of the Ethiopian Endod strain 44 (E-44) were compared for potency in the laboratory. Using the spray method,

Table I. The toxicity effectiveness berries crude extract of *Phytolacca dodecandra* against immature stage of Baetidae (Mayflies).

LC_{50} mg/l	181.94
LC_{50} LCL*	139.40
LC_{50} UCL*	222.57
LC_{90} mg/l	525.78
LC_{90} LCL*	413.12
LC_{90} UCL*	765.25
Chi Square (χ^2)	2.98

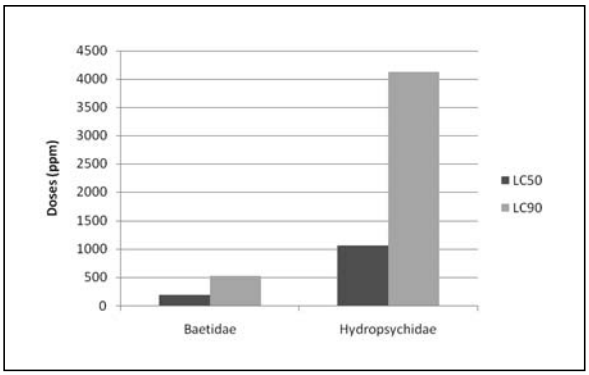

Figure 1. The toxicity effectiveness of berries crude extract of (Endod) *Phytolacca dodecandra* against immature stages of macroinvertebrates (Baetidae & Hydropsychidae).

100% snail mortality obtained. Snail mortality ranged from 20 to 100% using endod soap. There was a progressive decline in the snail population and infection in Bati stream compared with Worke stream, mainly due to sustained use of endod soap[5]. Aqueous extract of ground Endod (*Phytolacca dodecandra*) berries (type 44) was investigated for its cercariacidal and miracidiacidal properties. Assessment of cercariacidal activity of Endod berries indicated that mortality of cercariae exposed to aqueous extract of Endod berries increased with increase in concentration of the test material and exposure time[20]. In our present study, the percent of mortality rate was dose and exposure time dependent.

Saponin concentrations in water treated with an aqueous extract of *Phytolacca dodecandra* cultivar E44 were stable for 2 days then rapidly decreased during the third and fourth day. The saponin fraction extract of *Phytolacca dodecandra* was readily biodegraded (t1/2 = 15.8 h), and the complete consumption within a 10-day[21]. The above study indicates that an aqueous extract of *Phytolacca dodecandra* was biodegradable; therefore, its LC_{50} and LC_{90} values are extremely high in the present study. However, it shows that it has strong toxicity effectiveness against Baetidae than Hydropsychididae (Tables I, II and Figure 1).

Table II. The toxicity effectiveness berries crude extract of *Phytolacca dodecandra* against immature stage of Hydropsychididae (Caddisflies).

LC_{50} mg/l	1060.69
LC_{50} LCL*	789.44
LC_{50} UCL*	1443.20
LC_{90} mg/l	4120.40
LC_{90} LCL*	2613.24
LC_{90} UCL*	10534.53
Chi Square (χ^2)	1.72

Extracts of the fruit of *Phytolacca dodecandra* (Endod) demonstrate molluscicidal and other biological activities. Since, this plant is indigenous to some countries where schistosomiasis is a common problem and its use to control the snail vector. Thus, subject to the results of future safety assessment[22]. Several studies indicated that berries crude extract of *Phytolacca dodecandra* against schistosomiasis vector is effective and efficient. In spite of this, one must be taking into account in relation to their toxicity against aquatic macroinvertebrates and interruption of food chain in the aquatic ecosystem.

Obviously, berries of Endod (*Phytolacca dodecandra*) have been used as a source of medicine in Ethiopia since ancient time to treat several ailments and other numerous purposes such as washing clothes and to control fresh water snails by the rural community. However, the fact is remaining that the release of the Endod berries extract into the streams/rivers adversely affect the aquatic ecosystem specifically food chain. At the moment, in Africa particularly in Ethiopia conservation and sustainable use of aquatic resource have been a big challenge, mainly because of rapid population growth and associated activities like the discharge of domestic and industrial toxic wastes into the aquatic ecosystem. Macroinvertebrates are an important part of the aquatic food chain. Certainly, food chains are significant crucial key components in the aquatic ecosystem. Therefore, at this juncture preserving the food chain is extremely essential and inevitable.

References

1) Egziabher TBG. Diversity of the Ethiopian flora. In plant genetic resources of Ethiopia, ed. J.M.M. Engles, J.G. Hawkes, W. Melaku, Cambridge University Press, Cambridge; 1991.

2) Pankhurst R. An historical examination of traditional Ethiopian medicine and surgery. Ethiopian Med J 1965; 3: 157-172.

3) Wolde-Yohannes L, Demsbe T, Lambert JDH. "Cultivation studies of *Phytolacca dodecandra* and its role. In: Schistosomiasis control; in Endod II, L. Makhuba, A. Lemma, D. Heyneman, Eds. United Nations Council on International and Public Affairs, New York; 1987.

4) Kloos H, McCullough FS. Plant Molluscicides. In: A. Lemma, D. Heyneman, S.M. Silangwa, Eds. *Phytolacca dodecandra* (Endod) Dublin, Ireland: Tycooly International Publishing Ltd; 1984.

5) Abebe F, Erko B, Gemetchu T, Gundersen SG. Control of Biomphalaria pfeifferi population and schistosomiasis transmission in Ethiopia using the soap berry endod (*Phytolacca dodecandra*), with special emphasis on application methods. Trans R Soc Trop Med Hyg 2005; 99: 787-794.

6) Lemma A, Brody G, Newell GW, Parkhurst RM, Skinner WA. Endod (*Phytolacca dodecandra*), a natural product molluscicide: increased potency with butanol extraction. J Parasitol 1972; 58: 104-107.

7) Abate D, Fesseha S. In vitro and in vivo antifungal activity of endod *Phytolacca dodecandra*. Proceedings of a Workshop held in Nazareth, Ethiopia, 1994: May 2-4.

8) Lemma A. A preliminary report on the molluscicidal property of endod (*Phytolacca dodecandra*). Ethiopian Med J 1965; 3: 187-190.

9) Lemma A. Laboratory and field evaluation of the molluscicidal properties of *Phytolacca dodecandra*. Bull WHO 1970; 42: 597-617.

10) Lemma A. Present status of endod as a molluscicide for the control of schistosomiasis. Ethiopian Med J 1971; 9: 113-118.

11) Lemma A. Background and historical review. In: *Phytolacca dodecandra* (Endod) A. Lemma, D. Heyneman, S.M. Silangwa; Dublin, Ireland: Tycooly International Publishing Ltd 1984; 12-44.

12) Parkhurst RM, Thomas DW, Skinner WA, Cary LW. Molluscicidal saponins of *Phytolacca dodecandra*: oleanoglycotoxin-A. Phytochemistry 1973; 12: 1437-1442.

13) Esser KB, Semagn K, Wolde-Yohannes L. Medicinal use and social status of the soap berry endod (*Phytolacca dodecandra*) in Ethiopia. J Ethnopharmacol 2003; 85: 269-277.

14) Chinery M. Collins guide to the insects of Britain and Western Europe. A & C Black Publishers Ltd, United Kingdom; 2005.

15) Hickin NE. A useful reference to the larvae of the British Trichoptera is: "Caddis Larvae". Hutchinson & Co. Ltd London; 1967.

16) Jembere B. Evaluation of the toxicity potential of Milletia ferrginea (Hoechst) Baker against Sitophillus zeamais (Motsch.). Int J Pest Management 2002; 48(1): 29-32.

17) Finney DJ. Probit analysis. III edn. London: Cambridge University Press 1971; pp 1-333.

18) Abbott WS. A method for computing the effectiveness of the insecticide. J Econ Entomol 1925; 18: 265-267.

19) Stobaeus JK, Heath GE, Parkhurst RM, Jones WO, Webster JE. A laboratory study of the toxicity of the butanol extract of endod (*Phytolacca dodecandra*) on two species of freshwater fish and two species of aquatic snails. Vet Hum Toxicol 1990; 32: 212-216.

20) Birrie H, Balcha F, Erko B, Bezuneh A, Gemeda N. Investigation into the cercariacidal and miracidiacidal properties of Endod (*Phytolacca dodecandra*) berries (type 44). East Afr Med J 1998; 75: 311-314.

21) Mølgaard P, Chihaka A, Lemmich E, Furu P, Windberg C, Ingerslev F, Halling-Sørensen B. Biodegradability of the molluscicidal saponin of *Phytolacca dodecandra*. Regul Toxicol Pharmacol 2000; 32: 248-255.

22) Pezzuto JM, Swanson SM, Farnsworth NR. Evaluation of the mutagenic potential of endod (*Phytolacca dodecandra*), a molluscicide of potential value for the control of schistosomiasis. Toxicol Lett 1984; 22: 15-20.

Acknowledgements

The authors would like to acknowledge Jimma University Student Research Programme (SRP) for pursuing this research work by providing fund. Our last but not least heartfelt thanks go to our colleagues, School of Environmental Health Science, Faculty of Public Health, Jimma University, Jimma, Ethiopia, for their kind support and cooperation.