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Toxic effects of traditional Ethiopian fish poisoning plant *Milletia ferruginea* (Hochst) seed extract on aquatic macroinvertebrates

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Abstract. – The present investigation was carried out to evaluate the toxic effects of traditional Ethiopian fish poisoning plant Birbira [vernacular name (local native language, Amharic); *Milletia ferruginea*] seed extract on aquatic macroinvertebrates, Baetidae (Mayflies) and Hydropsychidae (Caddisflies), under laboratory conditions. In Ethiopia, toxic plant; *Milletia ferruginea* pulverized seeds have been used for fish poisoning since time immemorial. Macroinvertebrates are important biological indicators of alteration in the natural water sources. *Milletia ferruginea* seed extract was applied at concentrations of 125, 250, 500 1000 and 2000 ppm on Hydropsychididae whereas Baetidae were exposed at various concentrations viz., 31.25, 62.5, 125, 250 & 500 ppm. *Milletia ferruginea* seeds crude extract of lethal doses (LC_{50} and LC_{90}) required for Baetidae 49.29 mg/l and 172.52 mg/l were respectively and the respective doses (LC_{50} and LC_{90}) against Hydropsychidae were 679.64 mg/l and 2383.93 mg/l. The present investigation end result demonstrated that *Milletia ferruginea* seed extracts were extremely toxic to Baetidae than Hydropsychididae. As a result, application of *Milletia ferruginea* seed extracts into the rivers/streams for fish poisoning possibly leads to contamination and disruption of food chain in the aquatic ecosystem. Therefore, the concerned authorities should launch appropriate awareness campaign among the local inhabitants and fisherman about adverse effect of Birbira seed extracts. Furthermore, providing alternative ecofriendly techniques for fish harvesting may possibly bring constructive out come in the near future.

Key Words:

Plant extracts, *Milletia ferruginea*, Baetidae, Hydropsychidae, Ethiopia, Toxicity, Aquatic ecosystem, Macroinvertebrates, Birbira.

Introduction

Ethiopia is endowed with unique habitats that harbor many endemic species of plants. Of the 6500-7000 species of vascular plants in Ethiopia, 12% are endemic[1]. The country is well known for its significant geographical diversity that favors the formation of different habitat and vegetation zones[2]. Birbira, *Milletia ferruginea* (Hochst), is an indigenous plant species found only in Ethiopia. It is endemic to Ethiopia and widely distributed in the country. It occurs generally between 1000-2500 m above sea level and in the region where water is easily accessible such as streams or in rain forests.

The genus *Milletia* (Leguminosae, Papilonoideae) constitutes about 200 species in tropical and subtropical Africa, Asia and Australia[3]. Birbira is an indigenous tree in Ethiopia and, among other uses, it is commonly used as a fish toxicant[4-6]. Using poisons is an age-old method of capturing fish worldwide; the most commonly used chemical being rotenone[7]. This method has been exercised for ages are poisoning the fish with powders of birbira [*Milletia ferruginea* (Hochst) Baker] seeds[8]. Birbira is commonly found in areas where water is accessible, such as near streams or in rain forests. This simple easy access of fishermen to birbira too could have contributed to its wide application in fishing[6]. One of the sources for rotenone from Ethiopian plants is *Milletia ferruginea*[9]. Rotenone is highly toxic for aquatic life, especially to fish[10].

There are two sub-species known to occur in Ethiopia. These are: *Milletia f. ferruginea* which is confined to the northern part of the country and *Milletia f. darasana* which occurs in southern provinces, particularly Sidamo region[11].

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Trees from central and western Ethiopia show mixture of the two species[12]. The toxicity of the plant can be attributed of rotenone which is one of the dominant compounds found in the seed and stem bark of Birbira and is a well-known botanical insecticide through contact and stomach poisoning[13-15].

Toxicity effects of chemicals on aquatic organisms have been 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. 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[16].

Seed extracts of Birbira is extremely toxic to fishes as well environment. It is possibly affects other beneficial organisms in the aquatic ecosystem and ultimately disrupts the food chain due to their toxic nature. In addition, extensive studies have been carried out for their medicinal properties and so far only few limited number of studies have been focused on for their toxicity properties against aquatic beneficial organisms. Therefore, the present investigation was conducted to determine the toxic effects of traditional Ethiopian fish poisoning plant *Milletia ferruginea* (Hochst) seed extract on aquatic macroinvertebrates like Baetidae and Hydropsychidae, under the laboratory conditions.

Materials and Methods

Birbira 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. A plant species showing toxicity activities was selected; the species was Birbira. In Ethiopia, since the prehistoric eras the local rural inhabitants have been using this plant for medicinal and various other purposes. The collected voucher specimens were pressed, numbered, dried, identified and deposited at Jimma University Regional Herbarium. Identification of specimens was made with the help of herbarium materials, experts and taxonomic keys in the Flora of Ethiopia and Eritrea[17-19].

Birbira Plant Details

Toxonomic Position

Milletia ferruginea (Hochst.) Baker (1871)
Family: Leguminosae-papilionoideae
Subfamily: Papilionoideae
Genus: Milletia
Species: ferruginea

General Uses

The genus *Milletia* appears in the African pharmacopeia since centuries. It has a wide range of biological activities such as antitumoral, anti-inflammatory, antiviral, bacterial, insecticidal and pest-destroying. *Milletia* genus remains of a great importance in the traditional therapeutic arsenal[20]. *Milletia ferruginea* tree is used for fish poisoning where mature pod and seed are ground to fine powder and is spread over the surface of water[21]. Growing agricultural crops under *Milletia ferruginea,* a tree that is endemic to Ethiopia, is an age-old practice in the country. The soil near birbira tree is found to be rich in nutrients[22]. As a nitrogen-fixing species, birbira has been integrated with crops and coffee growing in Ethiopia. The seeds and roots of these plants are used as insecticides and piscicides in many parts of the world, and rotenone is responsible for their toxicity[23,24].

Physical Characteristics

The genus *Milletia* consist about 200 species. It is distributed in the tropical and subtropical countries belong to Africa, Asia and Australia. *M. ferruginea* (Amharic: "Birbira"; spelled as "Berbera" in Amha Bekele[24], "Brbrra" in MacLachlan[6]) is an indigenous plant species found only in Ethiopia. The tree is umbrella-shaped or flat-topped, and grows up to a height of 25 m[6] to 35 m[14]. The trunk of Birbira is mostly straight, with smooth grey bark. Short brown or golden hairs cover some surfaces of the flowers, fruits and leaves. Compound leaves up to 40 cm long; leaflets up to 9×2.5 cm; but smaller ones maybe as small as 3×1 cm. Leaflets generally are larger towards the end of the leaf. Flowers in groups up to 30 cm long including a stem of 5-10 cm. The flowers can be white to pink or rose in colour. The pods of the Birbira are large and sometimes open with a loud popping sound. Pod flat, up to 27×3 cm, with 5 to 10

seeds. They are about to about 1.3 to 1.7 cm across. The seed is dark red to orange colour, somewhat like a flattened disk or almost square with rounded corners[6].

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 section of the aquatic food chain and they are extremely sensitive to different types of chemical and physical conditions. Environmental modifications or pollution can alter macroinvertebrates communities. Macroinvertebrates are sampled in water bodies 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 contamination, toxicity, and pollution. Therefore, in this present study, the toxicity of *Milletia ferruginea* seed crude extract was evaluated against mayflies and caddisflies.

Scientific Classification

Baetidae (Mayflies)

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

Mayflies belong to the family called Baetidae. This family consists of nearly 900 described species and 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[25].

Scientific Classification

Hydropsychididae (Caddis flies)

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
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 caddis fly 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[26].

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 160 µS (microsiemen) respectively. Test organisms were collected by using macroinvertebrates 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.

Birbira Seed Collection and Extraction Preparation

Ripe seeds were collected from Birbira trees by accompanying fisherman, traditional users, translators and field assistants in outskirt of Jimma town, Ethiopia, in September 2008. The Authors also made observations in the field on the general habitats. Ripe seeds 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 into fine powder with locally made wooden mortar and pestle and 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[14].

Laboratory Evaluation for Toxicity Potential of *Milletia Ferruginea*

The toxicity of plant extracts on aquatic macroinvertebrates like Baetidae and Hydropsychidae were determined by using standard procedure. Testing seeds extract of Birbira for toxicity potential was carried out at different concentrations ranging from (Baetidae) 31.5 to 500 ppm and (Hydropsychididae) 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 plant 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 seed extracts of Birbira, 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 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[27]. The number of macro invertebrates in both control and test were recorded after 2/8 h of post treatment. Statistical analyses were performed with the SPSS 10.1 computer program (SPSS Inc. Chicago, Illinois, USA). When the control mortality was between 5-20 percent effects were corrected by using Abbott's formula[28].

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

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

Results

Toxicity of Ethiopian Indigenous plant *Milletia Ferruginea* Seeds Extract Against Aquatic Macroinvertebrates

The toxicity of *Milletia ferruginea* seed extract was tested against *Hydropsychidae* at different concentrations viz., 125, 250, 500 1000 & 2000 ppm and various parameters for the said effects are presented below in the Table II and Figure 1. Baetidae were exposed at various concentrations viz., 31.25, 62.5, 125, 250 & 500 ppm, since species of Baetidae extremely susceptible to seed crude extract of Birbira, and the mortality was recorded 2 h of post treatment (Table I and Figure 1).

The LC_{50} and LC_{90} values for seeds crude extract of *Milletia ferruginea* against Baetidae were 49.29 mg/l and 172.52 mg/l respectively for 2h. From these values as well as from Figure 1 and Table I it can be seen that Baetidae was extremely susceptible. The value of χ^2 was 7.81. Similarly, Table II and Figure 1 reveals that *Milletia ferruginea* seed extract of lethal doses (LC_{50} and

Table I. Toxicity Potential of *Milletia ferruginea* seeds extract against Baetidae (Mayflies).

LC_{50} mg/l	:49.29
LC_{50} LCL*	:30.85
LC_{50} UCL*	:67.18
LC_{90} mg/l	:172.52
LC_{90} LCL*	:120.22
LC_{90} UCL*	:340.97
Chi Square (χ^2)	:7.81

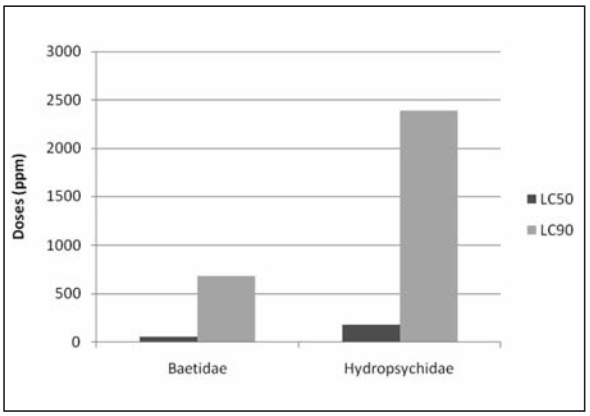

Figure 1. Toxicity potential of *Milletia ferruginea* seed extract against macroinvertebrates (Baetidae & Hydropsychidae).

LC_{90}) required for Hydropsychidae were 679.64 mg/l and 2383.93 mg/l respectively for 8 h. The value of χ^2 was 7.81 (Table II and Figure 1).

Discussion

The study clearly established that *Milletia ferruginea* (Birbira) seed extract was extremely potent against Baetidae with LC_{50} and LC_{90} were 49.29 mg/l and 172.52 mg/l respectively for 2 h (Figure 1 & Table I). LC_{50} and LC_{90} values of *Milletia ferruginea* (Birbira) seed extract against Baetidae is very less than Hydropsychidae and even the period of exposure was also particularly very shorter than the period of exposure of Hydropsychidae. A study conducted to test the toxicity potential of berries crude extract of *Phytolacca dodecandra* against Baetidae was observed and found that LC_{50} and LC_{90} values were 181.94 and 525.78 mg/l respectively[16]. In Ethiopia, the local rural inhabitants use berries crude extract of *Phytolacca dodecandra* for washing their cloths and to control the fresh water snails; those are acting as intermediate host for *Schistosoma* species, causative agent for Schistosomiasis.

Table II. Toxicity potential of *Milletia ferruginea* seeds extract against Hydropsychididae (Caddis flies).

LC_{50} mg/l	:679.64
LC_{50} LCL*	:507.84
LC_{50} UCL*	:940.86
LC_{90} mg/l.	:2383.93
LC_{90} LCL*	:1547.01
LC_{90} UCL*	:5286.46
Chi Square (χ^2)	:7.81

However, the LC_{50} and LC_{90} values of *Milletia ferruginea* (Birbira) seed extract were very low when compare with LC_{50} and LC_{90} values berries crude extract of *Phytolacca dodecandra* against Baetidae. Therefore, release of seed extract of Birbira into the river and stream is extremely danger to environment and other beneficial organisms in the aquatic ecosystem. Furthermore, the Baetidae more susceptible and exceedingly sensitive towards seed crude extract of *Milletia ferruginea* (Birbira) than Hydropsychidae. In addition, it also revealed that seed crude extract of *Milletia ferruginea* (Birbira) has strong lethal effect against Hydropsychidae with LC_{50} and LC_{90} were 679.64 mg/l and 2383.93 mg/l respectively for 8 h (Figure 1 & Table II). Similar result observed by previous study carried out in Ethiopia that that LC_{50} and LC_{90} values for berries crude extract of *Phytolacca dodecandra* against Hydropsychidae were 1060.69 and 4120.4 mg/l respectively[16].

A study indicated that toxicity effect of polar and non polar extracts of "Birbira", *Milletia ferruginea* seed powder were evaluated at different concentration levels (10- 40% w/v) against the different castes of adult *Macrotermes* termites and sorghum chaffer (*Pachnoda interrupta*) and compared with other plant extracts and standard insecticide. In the filter paper bioassays, water extract of *Milletia ferruginea* caused higher toxicity to all the castes of termites in which 93 to 100% mortality was recorded at all concentration levels. Water extract the Birbira seed (filtered with cheese cloth) caused 45-60% mortality sorghum chaffer within 24-48 hrs and this was significantly higher than mortality caused by the carbaryl, applied at recommended rate (1.5 kg/ 400 l of water)[11].

Toxicity potential of different plant parts of *Milletia ferruginea* (Hochst) Baker was tested against *Sitophilus zeamais* (Motsch.) in maize seeds and on filter paper. Polar solvents seed powder extracts were, however, significantly toxic. Among these, acetone extract was the most toxic extract and with the dose-response bioassay, LD_{50} = 65.45 mg per filter paper[14]. In addition, few studies well documented that Birbira seed extracts has the effective toxicity potential

against various type of insect pests[29,30]. Damte and Chichaybelu[33] were evaluated the toxicity of *Milletia* seed against Adzuki bean beetle, *Callsobruchus chinunesis*, Again, laboratory experiments found that the oils of *Milletia ferruginea* and *Azadirachta indica* were able to effectively control Adzuki bean beetle infestation of faba bean[31]. The present investigation found that the seed crude extract of *Milletia ferruginea* (Birbira) has strong toxicity against aquatic macroinvertebrates.

A crude chloroform extract of seeds of *Milletia dura* Dunn (Leguminosae) showed high activity (LC_{50} = 3.5 microg ml(-1) at 24 h) against second-instar larvae of the mosquito, *Aedes aegypti* L (Diptera: *Culicidae*). The rotenoids, deguelin and tephrosin, isolated from the seeds of this plant also showed potent activities, with LC_{50} values of 1.6 and 1.4 mg ml(-1) at 24 h, respectively[32]. The idea of neutralizing natural waters poisoned with Birbira (or rotenone) seems impracticable, as the detoxifying chemicals themselves could be harmful to the fish. For instance, it is known that chlorine/chloramine irritates the gills of fish and blocks oxygen carrying cells, leading to suffocation[33].

In any use of toxicants, adequate attention should be given to unwanted and even unexpected side effects. The use of toxicants in rivers is particularly fraught with danger because of the rapid downstream spread that is possible[7]. Obviously, Birbira seeds have been used as a source for numerous purposes by the rural community in Ethiopia. Particularly, local fisherman apply enormous amount of Birbira seed extract in the river as well streams for fish poisoning to collect fishes without any difficulty. The present investigation result demonstrated that Birbira seed extracts were extremely toxic and harmful to aquatic macroinvertebrates. Therefore, creating awareness among the local inhabitants and fisher man about adverse toxic effect of Birbira seed extracts and providing and introducing alternative technique to fisherman for fish harvesting may possibly pave the way to bring constructive outcome in the near future.

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