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TOXICITY STUDY OF *DIOSPYROS MONTANA* IN JUVENILE ZEBRA FISH

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ABSTRACT

Plants can be used to catch the fish. A study was conducted to evaluate the toxicity of fresh leaves of *Diospyros montana* in fish and to evaluate some phytochemical constituents. Fresh leaves were weighed, finely ground and constituted to 50 g/L in water. LC₅₀ study was conducted using Zebra-fish based on a preliminary range finding test. Serial concentrations were employed for the study *i.e.*, 0.5, 1.0, 1.5, 2.0 and 2.25 g/L, each concentration was tested in a container with 10 fishes in 4 liters of water to identify the LC₅₀ and it was found to be 1.37 g/L. Steroids and alkaloids were present in petroleum ether and methanol extracts respectively. Fresh leaves were negative for the presence of nitrate and cyanide on diphenylamine test and picrate paper test respectively. *Diospyros Montana* was toxic to fish, this property of the plant can be used to kill predatory fish in shrimp culture and pisciculture instead of ecotoxic chemicals.

Keywords: *Diospyros montana*, Zebra fish, LC₅₀, Nitrate, Cyanide, Phytochemistry.

INTRODUCTION

Since time immemorial man has used various techniques to catch fish for food purpose. Besides modern technology, many of the age old techniques are still in vogue in various parts of the world. *Diospyros Montana* Roxb. is one such plant used for catching fish by local folk in Western Ghats especially in rural areas of Sagar in Shimoga District of Karnataka State. [1] recorded various indigenous fish stupefying plants including *Diospyros montana* from western Maharashtra based on information given by the local natives during ethnobotanical expeditions. piscicidal property of the fresh aerial part of the *Mimosa pudica* plant was also explored. However, there is no experimental evidence on toxicity of *Diospyros montana* in fish. Therefore a study was conducted to evaluate toxicity of fresh leaves of *Diospyros montana* on fish and to analyse the methanol, chloroform, ether and aqueous extract for some phytochemical constituents.

MATERIALS AND METHODS

Fresh leaves of *Diospyros Montana* were collected from Botanical Garden, GKVK, UAS, Bangalore. One hundred gram of fresh leaves were weighed and finely ground and the volume was made to 2000 ml with distilled water (50 g/L) and was used as stock solution.

Juvenile Zebra fish (*Brachydaniorerio*) weighing less than 3 g were used in this study. Before and during the experiment the fish were kept in standard laboratory conditions in Central Laboratory, Karnataka State Pollution Control Board, Bangalore. LC₅₀ in fish was derived from the crushed fresh leaves of *Diospyros montana* as per Bioassay Method for evaluating acute toxicity of industrial effluents and waste water, IS 6582 (Part II), (2001).

Range finding toxicity test was conducted for fresh leaves, where, the concentrations of 0.5, 1.0, 2.5, 5, 6.25, 12.5, 25 and 50 g/L of fresh leaves were made from stock solution. Each concentration was tested on one fish in 100 ml of water along with one control with plain water. Fish were observed for 24 hours and death if any was recorded.

Based on range finding study, concentrations ranging from 0.5 to 2.25 g/L were considered to find the LC₅₀ of the plant leaves. Five serial concentrations were employed for the study *i.e.*, 0.5, 1.0, 1.5, 2.0 and 2.25 g/L. Each concentration was tested in a container with 10 fishes in 4 liters of water and observed for 48 hours for morbidity and mortality and the data generated was subjected to Probit analysis using GraphPad Prism 5 [2].

Fresh leaves were tested for presence of nitrate by Diphenylamine test [3] and cyanide by Picrate paper test [4]. Aqueous, ether, chloroform and methanol extracts were prepared according to standard extraction procedures [5]. Phytochemical analysis for screening of steroids, alkaloids, flavonoids, saponins, tannins and glycosides carried out by using thin layer chromatography [6].

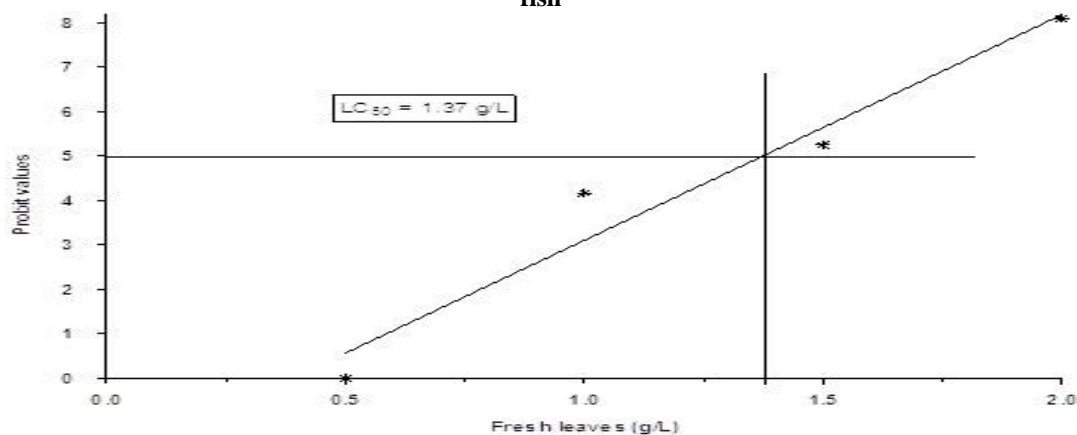
RESULTS AND DISCUSSION

The mortality was 100% in 2.25 g/L and 2.0 g/L, 60% in 1.5g/L, 20% in 1g/L and no death in 0.5g/L. LC₅₀ was 1.37g/L (Table 1, Fig. 1).

Table 1. Percentage death of fish and corresponding probit values at different concentrations of fresh leaves of *Diospyros Montana* and LC₅₀.

Concentration g/L	% Death	Probit values
0.5	0	0
1	20	4.16
1.5	60	5.25
2	100	8.09
2.25	100	8.09

Figure 1. Concentration of fresh leaves of *Diospyros montana* against probit values derived from percentage death of fish



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