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Rohitukine a potent insecticidal and pesticidal from *Dysoxylum binectariferum*

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ABSTRACT

Chloroform fraction of the ethanol extract of the stem bark of *D. binectariferum*, was evaluated for its larvicidal & insecticidal activities. Larvae of the yellow fever mosquito, *Aedes aegypti* L., & Housefly, *Musca domestica* L.. The results were found to be encouraging, to prove that the plant has a potential to be developed as a source of larvicidal & insecticidal principles.

KEYWORDS

Dysoxylum binectariferum;
Chloroform fraction;
Rohitukine;
Larvicidal;
Pesticidal.

INTRODUCTION

The genus *Dysoxylum*, belongs to the family Meliaceae, comprising of trees & higher plants. It is a large genus of about 200 species, of which a dozen occur in India, mainly in Bengal, Assam, South India & Andamans. The timber yielded by the trees has enormous economic importance[1]. The indigenous people have used many plants in the genus, as traditional medicine. *D. richii*, is such an example, which is used by indigenous Fijians as a traditional medicine to treat many diseases, including rigid limbs, facial distortion in children, lumps under the skin & other skin irritations, & as a remedy for sexually transmitted diseases. It is reportedly used as a remedy for fish poisoning & for convulsions[2]. The plant *D. binectariferum* is generally found occuring wild in the foothills of the Himalayas and Western Ghats in India. Apart from the Indian Subcontinent, the plant also occurs in other SouthEast Asian Countries, such as Burma, Thailand etc. The plant *D. binectariferum* is rich in a wide range of chemical compounds, such as Limonoids and alkaloids. Dysobinin, a tetranortriterpenoid, isolated from airdried powdered fruits of *D. binectariferum*, showed significant general CNS depressant action & mild antiinflammatory activity[3]. A chromone alkaloid from *D. binectari-ferum*, has proved to be a good Disease Modifying AntiRheumatic Agent, apart from having a CNS depressant action[4]. Present paper discusses the pesticidal and insecticidal biological activities of the Chloroform fraction, prepared from the crude Ethanolic extract of the plant on Mosquito larvae (*Aedes aegypti*) and Housefly (*Musca domestica*), respectively.

MATERIAL AND METHODS

Plant collection

The plant material was collected from Maharashtra in MayJune, 2005 and was authenticated by botany division of Lucknow University. The stem bark of the tree was collected from mature trees, air dried and finely grinded, prior to percolation in Ethanolic solvent, in the laboratory.

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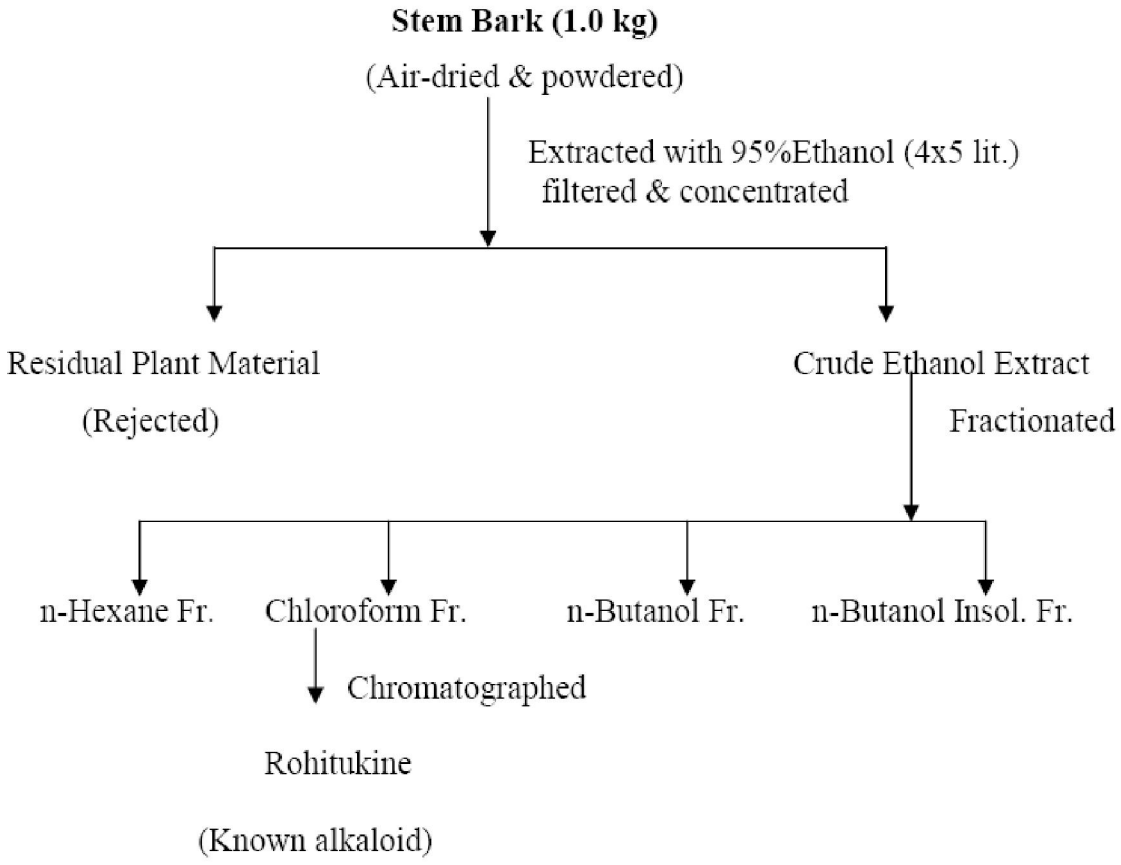
Extraction and fractionation

The airdried & powdered stem bark (1.0 kg), was subjected to extraction with distilled ethanol at room temperature, five times. The combined ethanolic extract was concentrated under reduced pressure below 50°C to a brown viscous mass, which was further dried under high vacuum to remove last traces of the solvent (wt. 50g). The ethanolic extract, thus obtained was further fractionated into four fractions, viz., hexane, chloroform, butanol, & butanol insoluble, fractions. The chloroform fraction on chromatography yielded a known alkaloid, along with a mixture of compounds. The alkaloid was identified as Rohitukine by physicochemical methods and cotlc with authentic sample.

Extraction and fractionation procedure



BIOASSAY METHODS

The rohitukine from the plant *D. binectariferum*, was dissolved in AR grade acetone to obtain the desired concentrations. For larvicidal action, early IV instar larvae of the yellow fever mosquito, *Aedes aegypti* were used. Colony of *Aedes aegypti* was maintained at 28± 2°C & 80% R.H. Dose range used was 0.12-0.25 ppm. The larvicidal activity was measured by placing twenty larvae in flasks containing test samples, which had been transferred by solution in acetone (0.1 ml) to water portions (1 ml), in which the larvae had been earlier placed. LC_{50} & LC_{95} were calculated through probit analysis. Similarly, the insecticidal activity was determined on a batch of twenty insects each, for five different doses ranging from 0.35 µg/insect to 5.75 µg/insect, in the increasing order of dosage/insect. A susceptible strain of housefly, *Musca domestica* L., reared away from any insecticide contamination were used for the purpose. The houseflies were treated topically on the dorsum of the abdomen with 1 µL of acetone containing the crude extract. Each batch of treated larvae & flies were placed in petridishes & held for 24 hours, at room temperature, and then the mortality percentage & LD_{50} & LD_{95} (for larvicidal activity), and LD_{50} & LD_{95}

(for insecticidal activity), were determined.

TESTING FOR PESTICIDAL AND INSECTICIDAL ACTIVITIES

The rohitukine from the chloroform fraction of the ccrude extract of *D. binectariferrum* was tested for insecticidal activity against *Musca domestica* (Housefly), and its larvicidal potential was determined against larvae of *Aedes aegypti*.

Larvicidal activity of rohitukine from *D. binectariferum* against mosquito (*Aedes aegypti*)

1 log Dose Plus 2.0; H=0.10; X1=5.0000; X2=6.2816

Concentration in ppm	No. of larvae used	Percentage Mortality
0.25	20.0	70
0.20	20.0	58
0.17	20.0	54
0.15	20.0	33
0.12	20.0	28

Log dose plus constant values: 1.398, 1.301, 1.230, 1.176, 1.079

Expected Probits	Working Probits	Improved Expected Probits	Final values of Log Dose Plus 2.0
5.548	5.528	5.558	1.390
5.194	5.205	5.201	1.302
4.936	5.101	4.940	1.274
4.738	5.565	4.740	1.129
4.384	4.420	4.383	1.089

Insecticidal activity of the rohitukine from *D. binectariferum* against housefly (*Musca domestica*)

Dose in µg/insect	No. of Insects used	Percentage Mortality
5.76	20.0	80
2.87	20.0	70
1.43	20.0	60
0.71	20.0	40
0.35	20.0	20

RESULTS AND DISCUSSION

Insecticidal (Larvicidal) activity

The larvicidal activity of the rohitukine was evaluated on the larvae of the yellow fever mosquito, *Aedes aegypti*. The solutions of different concentrations (expressed as ppm values) of the fraction, in decreasing order, were used on a set of 20 larvae each. 0.25 ppm was the lowest, while 0.12 ppm was the lowest concentration of the fraction used. The larval mortality at the 0.25 ppm dose was the highest at 70%, while it was the lowest at 28%, when the dose of the minimum concentration, i.e., 0.12 ppm was used.

A direct relationship was observed between the concentration of dose and the percentage larval mortality. Dosage values as expressed in log terms were, 1.398, 1.301, 1.230, 1.176 and 1.079 for 0.25, 0.20, 0.17, 0.15 and 0.12 ppm dose concentrations, respectively. Based on the results of the larvicidal activity, statistical probits were calculated. A good degree of agreement was found between the expected probits and working probits.

Log LC_{50} and Log LC_{95} values were calculated from LC_{50} and LC_{95} values. The LC_{50} and log LC_{50} values were comparatively better than their LC_{50} and LC_{95} counterparts. This was further confirmed after the comparison of parameters like variance, standard error, and upper and lower limits of these values.

Insecticidal activity

The insecticidal activity was determined on a batch of 20 insects each, for the five different doses ranging from 0.35 µg/insect (lowest dose) to 5.75 µg/insect (highest dose), in the increasing order of dosage/insect. The percentage mortality was found to increase with the corresponding increase in dosage, indicating a direct relationship between the two. Highest mortality of 80% was observed with 5.75 µg/insect, while the lowest mortality of 20% was found, when the dosage was minimum at 0.35 µg/insect. Based on the insecticidal data, statistical probits were calculated. The probit range indicated that the activity was moderate to good. Log Dose plus constant values came out to be 2.760, 2.458, 2.155, 1.85 and 1.544, for 5.75, 2.87, 1.43, 0.71 and 0.35 µg/insect doses respectively. The difference in the values of working probits and expected probits was less than that of the larvicidal activity. However, the final values of log dose plus 2.0 were higher than that of the larvicidal activity. The LD_{50} and LD_{95} and their log counterparts were higher than that reported under the larvicidal activity.

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