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Larvicidal Efficacy of Chloroform Extract of Mangrove Plant Acanthus Ilicofolius

B Suresh Kumar[1], Dr P Veerabramhachari[2]

[1]Research Scholar, Dept of Biosciences and Biotechnology, Krishna University, Machilipatnam
[2]Associate Professor, Department of Bio Sciences and Biotechnology, Krishna University, Machilipatnam.

sureshkbku2020@gmail.com

Abstract— Based on the data from Government of India National Vector Borne Disease Control Programme (NVBDCP) Department of Health & Family Welfare six species of local mosquitoes are identified viz, Anopheles culicifacies, Aedes aegypti, Culex quinquifasciatus, C. vishnui, C. pseudovishnui and C. tritaeniorhynchus. Based on the data sampling was done for Anopheles mosquito and their larvae, Aedes mosquito and their larvae, and Culex quinquifasciatus and their larvae. Acanthus ilicifolius, also known as holly-leaved acanthus, sea holly, and holy mangrove, is a shrub or herb belonging to the Acanthaceae family. The plant grows up to 2 meters (6 feet 7 inches) tall as a shrub. It has shallow tap roots and, on rare occasions, a stilt root. Kidney-shaped fruits. It can be found in mangrove areas. The leaves are collected, cleaned, and shade dried before being powdered and squeezed into a fine powder. Chloroform, and distilled water are used to macerate the powder. The stock solution is made with the extract. Bioassays using chloroform extract is used to examine the effect on larvae. For the control group, 100 percent mortality occurred during the first two hours, and for the 10% solution group, it occurred within six hours. The mortality rate for a 1 percent solution is 60% after 24 hours, but just 20% for a 0.1 percent solution. Based on the findings, it was determined that chloroform extract of Acanthus ilicofolius leaves can be utilized as larvicides.

Keywords— Mangrove, Acanthus, larvicides, mosquito larvae

Introduction

Mosquitoes are the major zoophilic vectors for the diseases like malaria, elephantiasis, encephalitis, dengue, chikun gunya etc. Major species that inhabit the localities of Machilipatnam are Anopheles culicifacies, Culex quinquifasciatus, Culex vishnui group and Aedes aegypti. (Tyagi et al., 2015) These Dipterans usually breed in stagnated water. Their life cycle is

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holometabolous. The most vulnerable stage for vector control is being the larval stage, which lasts for around fourteen days.

Mangroves, also known as "forest by the sea," are salt-tolerant plants that thrive in intertidal zones. Mangrove forests in Andhra Pradesh can be found in the Godavari and Krishna estuaries, and they are home to 35 different types of mangroves.

Mangroves are a group of flowering plants with strong adaptation and resilience to the extreme physico-chemical estuarine habitats found in tropical and subtropical countries. There are about three or more mangrove genera along with Acanthus. In tropical Asia and Africa, the genus Acanthus L. contains nearly 300 species, but only three are considered mangroves: Acanthus ilicifolius L., A. volubilis Wall., and A. ebracteatus Vahl. The genus name is derived from the Greek word 'Acantha,' which means thorn or thistle, and refers to the spiny leaves of some species. (Muktipada Panda, Rabindro Nath Samal, Susanta Nanda, Nikhil Lele, TVR Murthy, 2017)

Acanthus ilicifolius (Acanthaceae) has received a lot of attention because of its diverse secondary metabolites and traditional use in Indian and Chinese medicine. This plant has been identified as a mangrove. Steroids, triterpenoids, saponins, flavonoids, alkaloids, and tannins are abundant in these plants(Singh & Aeri, 2013). The plant has traditionally been used to treat dyspepsia, paralysis, asthma, headache, rheumatism, and skin diseases(Matos et al., 2022). The plant, known as 'Krishnasaireyaka' or 'Karimkurunji,' is one of nine plants associated with the Ayurvedic drug 'Sahachara,' which is used to treat rheumatic complaints. The plant's full potential has yet to be realised.

I. Methods

Plant sample is collected and shade dried. Dried leaves are ground, macerated in chloroform and stock solution is made. Following the WHO protocol for dose response of larvicides, the mosquito larvae are exposed to different concentrations for a 24 hours period of time, observations are made for every 4 hours. Using log probit regression analysis the LC50 and LC90 are calculated, ANOVA test is conducted to check statistical significance of the experiment done.(Satoto et al., 2013**)**

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Plant sample collection and chloroform extract preparation:

A sample of the Acanthus ilicofolius plant is taken. Under laboratory circumstances, the sample is shade-dried. Using a pestle and mortar, the separated leaves are ground. Chloroform is used to macerate the coarse powder. The mixture is filtered with filter paper after 24 hours of maceration. Allowed the filtrate to dry at room temperature. A 10% stock solution is made up of dried extract. The stock solution is serially diluted to 1 percent, 0.5 percent, 0.1 percent, 0.05 percent, and 0.01 percent solutions, as per the WHO procedure for larvicidal efficacy.

Collection of mosquito larvae:

Mosquito larvae of different mosquitoes and different instars are collected using plankton net from the surrounding stagnated water.

Conduction of Larvicidal bioassay:

Following the WHO protocol the larvicidal efficacy was tested(World Health Organization, 2005),(Borne et al., 2014). Ten larvae are transferred to each disposable cup and made up to 50ml with fresh water. Three replicates are prepared for each dilution is to be tested. Then appropriate volume of diluted chloroform extract of Acanthus ilicofolius is added to each cup. The entire setup is maintained under room temperature with normal lighting facility. Percentage of larval mortality is calculated by using Abbott's formula.

$$\text{Mortality \%} = (X\text{-}Y/X)\ 100$$

Where X = percentage survival in untreated control

Y = percentage survival in treated sample

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Table : Test results of the larvicidal bioassay test using the chloroform extract of Acanthus.ilicofolius against mosquito larvae after 24 hours

concentration	Total larvae tested	Total mortality	Percentage of larval mortality
1%	30	29	97%
0.5%	30	26	87%
0.1%	30	23	77%
0.05%	30	19	63%
0.01%	30	14	47%

Results and Discussion:

The final result of chloroform extract was analyzed using probit analysis and log-probit graph was plotted to determine the LC50 value of chloroform extract.

Table : log probit analysis of chloroform extract of Acanthus.ilicofolius against mosquito larvae after 24 hours.

conc/l	log of concentration	total dead	average	% mortality	PROBIT KILL
10000	4	29	9.7	97	6.88
5000.00	3.69	26	8.7	87	6.13
1000.00	3	23	7.7	77	5.74
500.00	2.70	19	6.3	63	5.33
100.00	2	14	4.7	47	4.92

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Graph: Graph plotted for probit kill

From the analysis LC50 value calculated is 154.8mg/dl and LC90 is 3890.5mg/dl as per EP17 A2. Multiple R value is above 0.95 indicates there is strong relationship between the variables. The significance f value is far less than 0.05, indicates there is least possibility of getting difference in obtained values. Hence the chloroform extract of Acanthus.ilicofolius has lethal effect on the mosquito larvae. It is more abundant in the mangroves and can be used as a potent larvicide as the larvae became fragile after 24hrs of exposure.

Conclusion:

The chloroform extract of Acanthus ilicofolius had the larvicidal activity against mosquito larvae.

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