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## Mosquito larvicidal efficacy of leaf extract from mangrove plant *Rhizophora mucronata* (Family: Rhizophoraceae) against *Anopheles* and *Aedes* species

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**ABSTRACT**

The crude extract of mangrove plant *Rhizophora mucronata* was prepared in this present work. The extract was testified for its anti-mosquito larvicidal property against two species of mosquito viz: *Anopheles stephensi* and *Aedes aegypti*. The 48 hours exposure of various dilutions of 100, 200, 300, 400, 500 ppm were given to the III to IV instar larval population. Percentage mortality data was analyzed statistically and compared. The larva of *Anopheles stephensi* was more susceptible than *Aedes aegypti*. This concept was substantiated by comparing the LC<sub>50</sub> values of IIIrd instar larva of both species. The results of histochemical demonstration for acetyl cholinesterase in supra esophageal ganglion had revealed the inhibition of acetyl cholinesterase by the treatments of plant extract. The above results are discussed with the advantages of bioactive anti mosquito larval compound.

**Keywords:** Anti mosquito larvicide; larvicidal principle from mangrove plant; acetyl cholinesterase inhibition by mangrove plant extract; pesticidal value of mangrove plant extract.

**1. Introduction**

Mangrove forest normally grows in tropical climates. It requires uniform warm climatic state for survival and development. The vast floral resources of mangrove forest are best known for their medicinal properties. Studies pertaining to the medicinal relevance of mangrove forest plants and their bioactive compounds have been increasing consistently in/ these days. The application of mangrove plant extracts for the treatment of health disorders has been practiced for many centuries as a common method. The mangrove plants and their constitutional metabolites are biologically unparalleled. The derived biochemical substances of mangrove plants are unique in their reactions. Since they possess competence in many bioactive principles against disease producing microbial organisms. Much interest has been shown by research workers in recent days. Many antimicrobial metabolites are extracted and displayed for their antibiotic reactions on many pathogenic species of genus *Shigella*, *Staphylococcus*, *Escherichia* and *Penicillium*. The recent review by Piyusha *et al.* [1] gives an exhaustive account of biological compounds of mangrove community plants and their active antibacterial, antiviral, antifungal and anthelmintic properties. Nevertheless apart from medicinal values mangrove plants also have rich resources of steroids, terpenes, saponins, flavonoids, alkaloids and tannins.

Throughout the world, mosquitoes are responsible for the transmission of several human diseases. WHO has described the mosquito as "public enemy number one" and reported mosquito borne diseases across globally infecting more than 700,000,000 people every year. In Indian scenario the data is alarming and gives that about 40,000,000 individuals are affected by this arthropod vector per year. Mosquitoes are passing pathogens of life threatening diseases like malaria, yellow fever, dengue, chikungunya, filariasis and encephalitis.

Mosquito control is a necessary measure to improve environmental quality and public health. The controlling strategies are largely by synthetic chemical substances. The chief anti mosquito insecticides are organochlorine and organophosphate compounds. The spray or application of chemicals can pose many environmental sustainable problems such as harmful effect to human beings, toxic to non-target organisms and the nature of non-degradable compositions. Besides, the pesticidal residues enter into ecosystem, through food web it circulates and biomagnifies.

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The alternative method of controlling mosquito with eco-friendly approach has been appeared after the implementation of Environmental Protection Act to check the spraying of chemical agents in natural environment. Keeping in view of this alternative controlling methods, many research workers have been paying their attention to develop a safer anti-mosquito bio-compound of plant origin. Identification, isolation and mass synthesis of bioactive principle against mosquito menace are imperative for the management of mosquito borne diseases.

The thorough scanning of literature tells that current state of knowledge on phytochemicals which proved for larvicidal action is available in the review article of Shallan *et al.* [2]. *Rhizophora mucronata* is a mangrove ever green tree that grows on the river banks and on the edge of sea water. It has a large number of aerial stilt roots. The habitat of this tree is estuaries and are more tolerant of changing climate. It is a native of India. The various parts of the tree are used as folk medicine from time immemorial. The present study is an attempt to extract anti mosquito larvicidal bioactive principle and observe its toxic action on larval biology.

## 2. Materials and Methods

The materials of the present study were leaves of *Rhizophora mucronata*, a mangrove tree (Family: Rhizophoraceae) collected from the "Kadalundi" estuarine waters located near Kannoor, Kerala State, India. The materials also included the larval population of *Anopheles stephensi* and *Aedes aegypti* mosquito species. The eggs were collected from National Centre for Disease Control, Field station, Mettupalayam, Tamil Nadu, India. The eggs were brought to the laboratory and transferred to 18 x 13 x 4 cm plastic trays containing 500 ml of water for hatching. The mosquito larvae were fed by pedigre biscuits and yeast at 3:1 ratio.

### 2.1 Maintenance of mosquito larva

The larvae were maintained at  $27 \pm 2$  °C, 75-85% relative humidity under photoperiod of 14:10 (light/dark).

### 2.2 Histochemical technique

The localization of acetylcholinesterase (here in called as Ache) in 'brain' tissue was determined by histochemical procedure as described by Patrick *et al.* [3]. The tissue samples were processed in Sipcon Model Sp-40 automatic tissue processor, India. Serial microtome (Leica Microtome, India) cut sections of mosquito larval heads at 5 µm were used. The sections were incubated with the substrate acetylthiocholine iodide for 1 hour. The non-specific cholinesterase substances were inhibited by treating the sections with diethyl phenothiazine hydrochloride. Incubation of the chromogenic agent for acetylcholinesterase DTNB for 1 hour was given prior to staining the sections with haematoxylin and eosin to know the reaction of Ache. The sections were mounted, viewed

under microscope and photographed by Digi Eye Digital Microscope camera (Dewinter, Germany).

### 2.3 Extraction of Phytochemical

The collected leaves were air dried in room temperature for 5 days. The dried leaves were made into fine powder state by grinding in blender. The extraction was done with methanol by Soxhlet apparatus. The crude was kept at 4 °C for further use.

### 2.4 Larval toxicity test

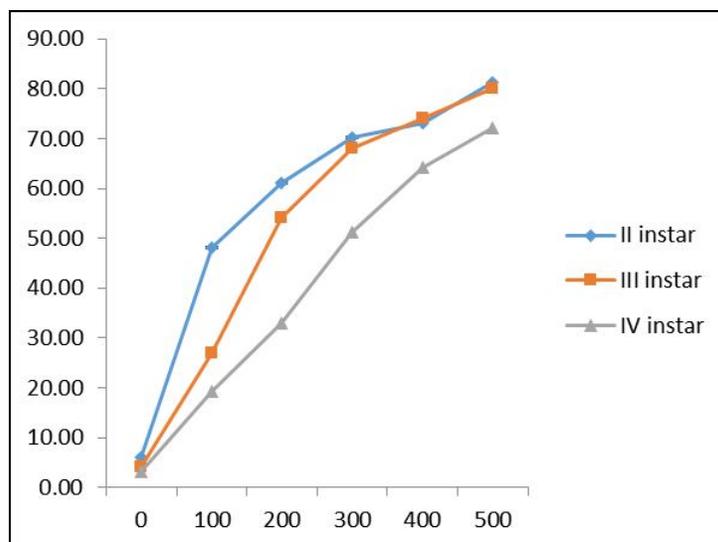
The laboratory colonies of mosquito larva of two species under investigation were used for the efficacy of the larvicidal action of the extract prepared as above. In each case of the species, 100 numbers of larva of II to IV instar stages were introduced into 500 ml tray containing of dechlorinated double distilled water and the crude extract of *Rhizophora mucronata*. Standard method of testing the susceptibility of mosquito larva was followed in all the category of observations. The methanol leaf extract was utilized at 100, 200, 300, 400 and 500 ppm dilutions in bioassays against larval instars of two mosquito species. Routine larval food was given during the experimental period. In each larval category and test concentration 3 replicates were observed. The parallel control batch was also maintained. Mortality rate was calculated at 48 hrs. of exposure. The mortality rates were tabulated and analyzed statistically for the toxicological examinations on brain tissues, the 48 hour treatment of critical concentration (LC<sub>50</sub>) on III instar larva of both species were given prior to the tissue process. The usual log-probit analysis with Yeat's control correction factor was followed for the determination of LC<sub>50</sub> values.

## 3. Results

Table 1 is furnished with data obtained for *Anopheles stephensi*. From the table it is evident that the control unexposed larval batches of II, III and IV instars population had the mortality percentage units as  $6.01 \pm 0.16$ ;  $4.5 \pm 0.12$  and  $3.11 \pm 0.08$ , respectively. On the other hand in experimental studies with the exposure of plant extract were exhibited gradual increase of mortality percentages. At 100 ppm treatments the mortality percentage for II, III and IV instars the recorded units were  $48.16 \pm 0.251$ ;  $27.01 \pm$  and  $19.18 \pm 0.381$ . The corresponding values at 500 ppm were the maximum mortality rate of  $81.2 \pm$ ;  $80.15 \pm 0.136$  and  $72.08 \pm 0.144$ . There were many intermediate levels of mortality percentages in ranges between 100 ppm and 500 ppm. There could be seen a gradual increase of mortality rate. The comparison among three instars larval population had disclosed that second instar was more susceptible to the plant extract. Figure 1 is the graph plotted with the data for above observations. It is also indicative for the susceptible trend due to the plant extract exposures at various dilutions among three different developmental stages of mosquito *Anopheles stephensi* (Table 1 and Fig. 1).

**Table 1:** Percentage mortality of *Anopheles stephensi* mosquito larva upon exposure of leaf extract from *Rhizophora mucronata* after 48 Hr. at different concentrations.

|            | 0               | 100               | 200               | 300               | 400               | 500               |
|------------|-----------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| II instar  | $6.01 \pm 0.16$ | $48.16 \pm 0.251$ | $61.11 \pm 0.311$ | $70.19 \pm 0.333$ | $73.18 \pm 0.161$ | $81.2 \pm 0.148$  |
| III instar | $4.15 \pm 0.12$ | $27.01 \pm 0.344$ | $54.09 \pm 0.511$ | $68.11 \pm 0.111$ | $74.15 \pm 0.133$ | $80.15 \pm 0.136$ |
| IV instar  | $3.11 \pm 0.08$ | $19.18 \pm 0.381$ | $33.08 \pm 0.172$ | $51.14 \pm 0.017$ | $64.11 \pm 0.33$  | $72.08 \pm 0.144$ |

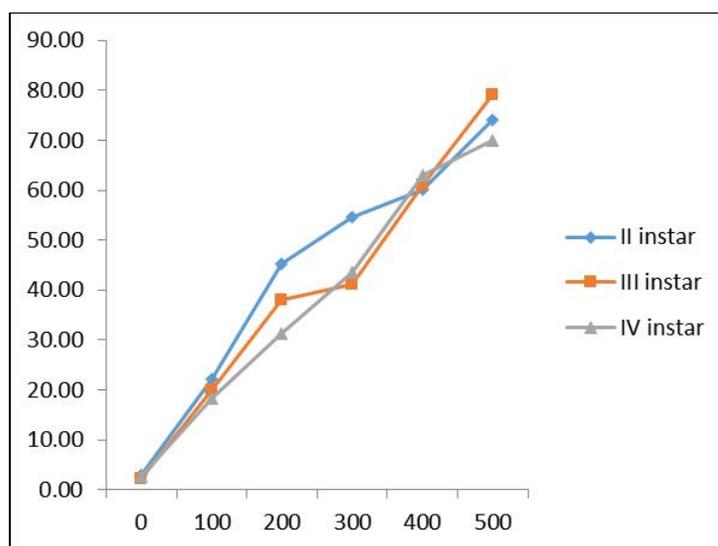


**Fig 1:** Percentage mortality of *Anopheles stephensi* mosquito larva upon exposure of leaf extract from *Rhizophora mucronata* after 48 Hr. at different concentrations.

Table 2 is presented with the data collected for the *Aedes aegypti*. From the table data it is also evident that crude extract from the leaves of *Rhizophora mucronata* exhibited a series of mortality rate upon various dilutions viz: 100 ppm; 200 ppm; 300 ppm; 400 ppm and 500 ppm among three different larval stages of *Aedes aegypti*. The mortality percentage data for control groups of II, III and IV instars, the values were  $3.01 \pm 0.019$ ;  $2.31 \pm 0.115$  and  $2.6 \pm 0.018$ . The equal data for experimental trails had shown an elevated level of mortality rates. There was a presence of steady/ increased mortality units as that of *Anopheles stephensi*. (Table 2 and Fig. 2). However at exposures of 500 ppm experiments, the three larval stages had the mortality percent of  $74.07 \pm 0.199$ ;  $79.01 \pm 0.512$  and  $70.06 \pm 0.138$  respective for II, III and IV instars. The corresponding records in the case of former mosquito *Anopheles stephensi* were higher (Table 1). This suggested that *Aedes aegypti* has less susceptibility to the plant extract under investigation.

This concept was substantiated on comparison of the  $LC_{50}$  values for III instar larva of both mosquito species. The log-probit mortality analysis yielded the  $LC_{50}$  critical concentration as 347.5 ppm for *Aedes aegypti* (The lower 95% fiducial limit is 10.619; the upper limit is 13.401) and 190.5 ppm for *Anopheles stephensi* (The lower 95% fiducial limit is 6.522; the upper limit is 3.245). The above forgoing results unambiguously suggested that the crude leaves methanol extract of *Rhizophora mucronata* has the antimosquito biocompound and its potential to eradicate the larval population.

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**Fig 2:** Percentage mortality of *Aedes aegypti* mosquito larva upon exposure of leaf extract of *Rhizophora mucronata* after 48 Hr at different concentrations.

**Table 2:** Percentage mortality of *Aedes aegypti* mosquito larva upon exposure of leaf extract of *Rhizophora mucronata* after 48 Hr at different concentrations.

|            | 0          | 100         | 200         | 300         | 400         | 500         |
|------------|------------|-------------|-------------|-------------|-------------|-------------|
| II instar  | 3.01±0.019 | 22.11±0.179 | 45.16±0.212 | 54.51±0.519 | 60.03±0.051 | 74.07±0.189 |
| III instar | 2.31±0.115 | 20.11±0.511 | 38.12±0.081 | 41.15±0.199 | 61.11±0.158 | 79.01±0.512 |
| IV instar  | 2.6±0.018  | 18.22±0.156 | 31.35±0.374 | 43.66±0.079 | 63.12±0.333 | 70.06±0.138 |

A common morphological and behavioral aspects in the larva of two mosquito species were also examined in this present work. It was noted that larvae gradually became inactive within few hours of exposure. The microscopic examination of dead larva revealed a change in the integument molting. There was an evidence for cuticle tissue chitin disintegration. In prolonged treatments beyond 10 hours several larva have shown unusual stretching of body particularly in thoracic regions (Fig. 3 and 4).

This would allow to presume the effect of neuro endocrinal system of mosquito species under examination. As many antimosquito preparations of natural origins and chemical pesticides cause functional damage of the nervous system. The Ache is the significant neuro secretory substance for the signal transmission. Therefore in this present study an attempt also has been made to know the activity of Ache in the oesophageal nerve ganglion by histochemically to ascertain whether the crude extract of *Rhizophora mucronata* has any influence on the neuro endocrinal signals. The same has been worked out and the results are presented in Table 3. The substrate acetylcholine iodide incubation

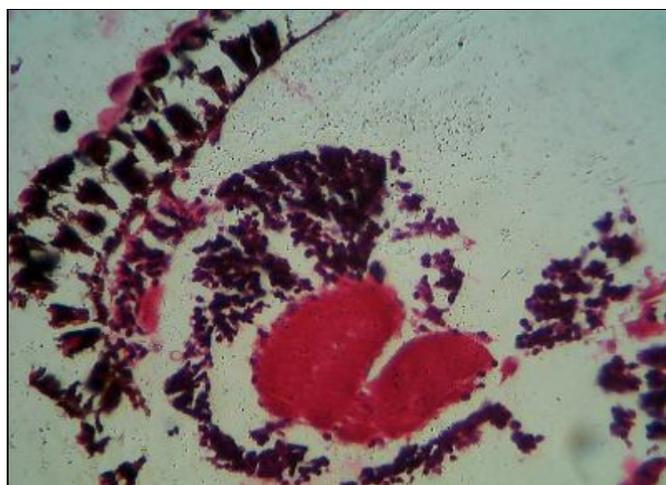
displayed intensive positive (+++) reaction in control tissue section. This would suggest the presence of cholinesterase activity in ganglion. The staining reaction for control tissue gave positive (+) reaction, however it was less intensive for cholinesterase demonstration. Cholinesterase normally includes the activity of non-specific cholinesterase. To remove these non-specific cholinesterase for the acetylcholinesterase identity diethyl amino prophyll pheno thiazine hydrochloride was used as an inhibitor. The same test had shown highly positive (++) for the presence of acetylcholinesterase in nerve mass of both control as well as experimental. The tissue section after this examination was stained by dithio nitro benzoic acid (DTNB) a coloring agent for Ache. The results were exhibited significantly less positive reactions (+) in experimental tissues against equal control sections which had shown highly intensive positive reaction for Ache. The deserving point to be noted in this context is that when the above tissues stained by haematoxylin and eosin following Ache reaction hold forth for the presence of abnormal neural plexues in ganglion of extract treated species. (Table 3 and Figs. 3 to 5).

**Table 3:** Histochemical determination of Acetylcholinesterase in esophageal ganglion "brain" in mosquito larva of *Anopheles stephensi* and *Aedes aegypti*.

| Stains  | Activity  | Reaction |              |
|---|---|----------|--------------|
|   |   | Control  | Experimental |
| Acetylthiocholine iodide                            | Cholinesterase binding agent  | +++      | +            |
| Diethyl amino prophyll pheno thiazine hydrochloride | Inhibition of non-specific cholinesterase   | ++       | ++           |
| Dithio nitro benzoic acid (DTNB)                    | Chromogenic agent - reddish brown precipitation for the presence of acetyl cholinesterase | +++      | +            |
| Haemotoxylin + eosin                                | Abnormal nerve plexues  | -        | +++          |

+++ : highly positive, ++ : Positive, + : Low positive, - Negative (no reaction)

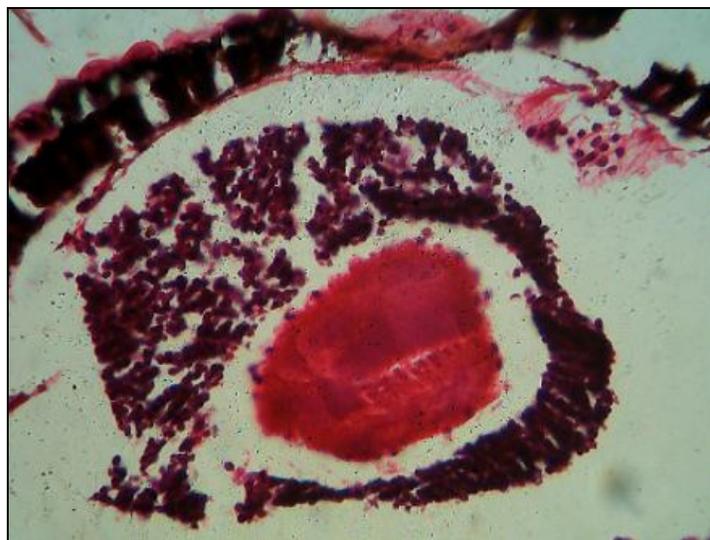
*Anopheles stephensi*

**Fig 3:** Photomicrograph of esophageal ganglion of III instar larva of *Anopheles stephensi* stained for histochemical reaction for the presence of Ache inhibition (magnification 45 x 10).

In the light of above foregoing histochemical examinations, it is reasonable to assume the inhibition of Ache in neuro endocrine nerve ganglion of mosquito by the extract from *Rhizophora mucronata*. It is also presumed that the extract has bioactive

principles against mosquito larva and impairing neuro endocrinal regulation by the inhibition of neuro transmitting signal molecules namely Ache.

*Aedes aegypti*



**Fig 4:** Photomicrograph of esophageal ganglion of III instar larva of *Aedes aegypti* stained for histochemical reaction for the presence of Ache inhibition (magnification 45 x 10).



**Fig 5:** Photomicrograph of mosquito experimental larval esophageal ganglion showing abnormal nerve plexuses stained after haemotoxylin and eosin (magnification 45 x 10).

#### 4. Discussion

Mosquitoes act as vector for many life threatening diseases. Now much special attention is given for alternative eco-friendly methods in mosquito control programmes. When the continuous spraying of synthetic chemical pesticides develops resistance, Plant possess numerous bioactive substances, which may exhibit biopesticidal properties. The efficacy of photochemical molecules against mosquito in particular the developing larval stages depends on the nature of plant species, part used, method of extraction and the toxic doses. A thorough review on this subject is available in the recent article written by Anupam Ghosh et al. [4].

The results of present investigation on the mosquito larvicidal efficacy by the methanol extract from the leaves of *Rhizophora mucronata*, a mangrove (loop-root red plant) tree sounding a clear demonstration. The different susceptible action of various larval stages of two mosquito species is reported in this present study. Similar differences in the mortality due to dose dependent exposure of crude extracts from various other plant species are also shown by earlier workers (Kaushik and Siaini, [5]; Singh *et al.* [6]; Srivastava *et al.* [7]).

The most meritorious point to be deserved in this present work is the histochemical observations that have exhibited the inhibition of Ache in oesophageal nerve ganglion of experimental mosquito

larval group. Ache is a significant biomolecule of neuro signal transmission. For the normal metamorphosis and development of the mosquito larval stages the signal pathway is essential. Ache influences the molting of the insect integument by the hormone ecdysone secreted by the neuro endocrinal system. The crude methanol extract *Rhizophora mucronata* and its treatment would suggest the functional damage of corpora cardiaca and corpora allata (Thoracic endocrine glands of insects) through the inhibition of Ache. It is suggested that extract could be selected as bioactive toxic phytochemical for the control of mosquito population.

## 5. Conclusion

Environmental safety is the predominant talk of today. Using phytochemical for the eradication of mosquito larva may reduce the dependency of toxic chemicals. The ethano pharmaco approach may render utilization of local resources, local employment opportunities and reduce the economic burden. The combined application of pesticide of biological origin and predators may reduce the burden of mosquito control and the risk of vector borne diseases. It is desirable to undertake further research to isolate and identify the active ingredient of the crude extract derived from *Rhizophora mucronata*.

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