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Research Article

Qualitative Phytochemical Tests and Bioactivity of *Vitex negundo* L. Leaf extracts against *Aedes aegypti* L.

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Abstract

Qualitative Phytochemical tests and bioactivity of aqueous, acetone and ethanolic leaf extracts of *Vitex negundo* L. against *Aedes aegypti* L. mosquito larvae were carried out. Alkaloid, carbohydrate, coumarin, flavonoid, phenol, tannin and triterpenoid were located in both acetone and ethanol extract except carbohydrate in acetone extract. Alkaloid was absent in aqueous extract. Anthocyanin, anthraquinone and protein were absent in all the extracts studied. For mosquito larvicidal activity *Aedes aegypti* L. 4th Instar larvae were selected and the mortality percentage was calculated and compared. Different concentrations of aqueous, acetone and ethanol extracts (1mg/ml, 3mg/ml and 5mg/ml) with different time of exposure (24, 48 and 72 hours) were evaluated and the results showed that in aqueous extract mortality percentage was obtained (20%) only on 5mg/ml on exposure to 72 hours. Mortality percentage was zero in 1mg/ml and 3mg/ml on exposure to 24 and 48 hours. The highest mortality percentage (100%) was obtained both in acetone and ethanol extracts with concentrations of 3mg/ml and 5mg/ml on exposure to 48 and 72 hours respectively. The results revealed that mortality percentage was increases with increase in concentration as well as time of exposure in all the extracts studied.

Keywords: *Vitex negundo*, *Aedes aegypti*, Phytochemical tests, Mosquito larvicidal activity.

1. Introduction

Vitex negundo L commonly known as *Karinochi* (Malayalam) or *Nirgundi* (Hindi) (Plate No.1) belongs to the family Verbenaceae. The plant is well-known for its medicinal properties. Leaves are reported to have antibacterial, laxative, antioxidant, anticonvulsant, hypoglycaemic and anti-inflammatory properties. Pesticidal and antifungal properties of this plant are also reported [1]. Leaves as a paste, used for inflammatory swellings of the joints formed due to rheumatism, hydrocele and splenic enlargement. Effect of plant extracts or essential oils against mosquito larvae was confirmed by many researchers. Larvicidal activity of *Vitex negundo* L against *Culex quinquefasciatus* mosquito have been reported [2]. Previous studies have shown that diseases such as malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis are transmitted through vectors [3]. Mosquitoes are the major vectors for these diseases. Nowadays Dengue fever is very common in our localities, it can be controlled only by killing the mosquito larvae. Synthetic larvicides are toxic to humans and other useful organisms. Hence in search of an eco-friendly plant-based source as a larvicide, in the present investigation *Vitex negundo* L leaves were selected and analysed.

Plate No. 1: *Vitex negundo* L Habit and Flowering Twig

2.Materials and Methods

Plant materials for the present study such as leaves of *Vitex negundo* L collected from Pullumedu, Udumbanchola Thaluk of Idukki District. The collected plant materials were brought to the laboratory and the identification was confirmed by using Flora of Presidency of Madras [4] and flowering plants of Kerala [5].

2.1 Preparation of crude extract

Fresh leaves were cleaned, shade dried and completely dried in an oven at 60°C for overnight. It was coarsely powdered, **(Plate No.2)** stored in air tight polythene bag. Phytochemical tests were conducted using aqueous, acetone and ethanol extracts. For Phytochemical tests 20 gram of powdered material was extracted with 200 ml of each of the solvent and kept it for 48 hours with occasional shaking. The extraction was carried out in room temperature **(Plate No.3)**. After 48 hours, extracts were filtered through Whatman No.1 filter paper and concentrated. Phytochemical tests were conducted on all the three extracts by using standard methods [6 - 9].

Plate No.2: Dried Leaf Powder of *Vitex negundo* L. Plate No. 3: Aqueous, Acetone and Ethanol Extracts of *Vitex negundo* L. Leaves

2.2 Qualitative Phytochemical Tests

Phytochemical tests were conducted on all the three extracts and the results are represented on **Table No. 1**

Test for Alkaloid

2 ml of concentrated HCI was added to 2ml of extract followed by the addition of few drops of Mayer's Reagent. Formation of green colour or white precipitate indicates the presence of alkaloids.

Test for Anthocyanin

Add 1 ml of 2 N sodium hydroxide to 1ml of the extract and heated for 5 minutes at 100 °C. Formation of bluish green colour indicates the presence of anthocyanin.

Test for Anthraquinone

Few drops of 2% HCl were added to 1 ml of the extract. Formation of red precipitate indicates the presence of anthraquinone.

Test for Carbohydrate

Benedict’s reagent was added to 1ml of extract and heated for 2 minutes. A red brown precipitate indicates the presence of monosaccharaides.

Test for Coumarins

1ml of 10% sodium hydroxide was added to 1ml of extract. Formation of yellow colour indicates the presence of coumarins.

Test for Flavonoids

1ml of 2N sodium hydroxide was added to 2ml of extract. Yellow colour was obtained that indicates the presence of flavonoids.

Test for Phenols

To 1ml of extract, add 2 ml of distilled water followed by the addition of few drops of 10% ferric chloride. Formation of blue or green colour indicates the presence of phenols.

Test for Protein

1 drop of 2% copper sulphate solution was treated with 2ml of extract. To this 1ml of ethanol was added followed by an excess of potassium hydroxide pellets. Pink colour layer indicates the presence of proteins.

Test for Tannin

Add 2ml of 5% ferric chloride to 1ml of the extract. Formation of dark blue or greenish black colour indicates the presence of tannins.

Test for Triterpenoid

2 ml of chloroform and concentrated H_2SO_4 was treated with 0.5ml of the extract. Formation of red brown colour indicates the presence of Triterpenoid.

3. Mosquito Larvicidal Activity

3.1 (a) Collection and Identification of Larvae

Larvicidal activity was conducted against 4th Instar larvae of *Aedes aegypti* L. The larvae were collected from the bucket kept opened in nearby area of Herbal Garden in the campus of St. Dominic's college Kanjirapally. The bucket contained full of rain water with abundant growth of mosquito larvae. The larvae were collected and transferred to plastic tray and brought to Zoology laboratory and identified. Identification of the mosquito larval stage was done by Ms. Sruthy Pekson, Department of Zoology, St. Dominic's College Kanjirapally. Photo-micrographs of mosquito larvae were taken using Olympus CX43Microscope fitted with Magcam DC5 camera. The images were analysed using Magvision software and presented on **(Plate No: 3 (i), (ii) and (iii)**).

3.1 (b) Larvae of *Aedes aegypti*

Aedes aegypti larvae resemble other mosquito larvae in their morphology; in general, they have an ovoid head, thorax and abdomen of nine segments. The posterior segment (anal) has four lobed gills for osmotic regulation and a short barrel-shaped siphon bearing a single pair of sub-ventral tufts for breathing at the water surface [10-12]. Additional morphologic characteristics include at least three pairs of setae in the ventral brush, antennae that are not greatly flattened, and a lack of enormous setae on the thorax. The body of an adult *Aedes aegypti* mosquito is composed of head, thorax, and abdomen. *Aedes aegypti* males and females are similar in appearance except for the differences in size and form of the antennae (males have plumose antennae), maxillary palps (females have shorter palps), abdomen, claws and in scale markings [13]. Growth and development of larval instars is temperature dependent, however, complex interactions with other factors such as resource availability and intraspecific density also contribute to variation in development rate [14]. At cool environmental

temperatures (around 15 °C), *Aedes aegypti* larvae can remain in a particular instar for months, so long as the water supply is sufficient [15,16].

3.2 Preparation of Crude extracts for Larvicidal Activity

10-gram dried leaf powder of *Vitex negundo* L. was extracted separately in water, acetone and ethanol at room temperature for 48hrs with occasional shaking. Extracts were filtered by using Whatman No.1 filter paper. The filtrates were concentrated to dryness in water bath.

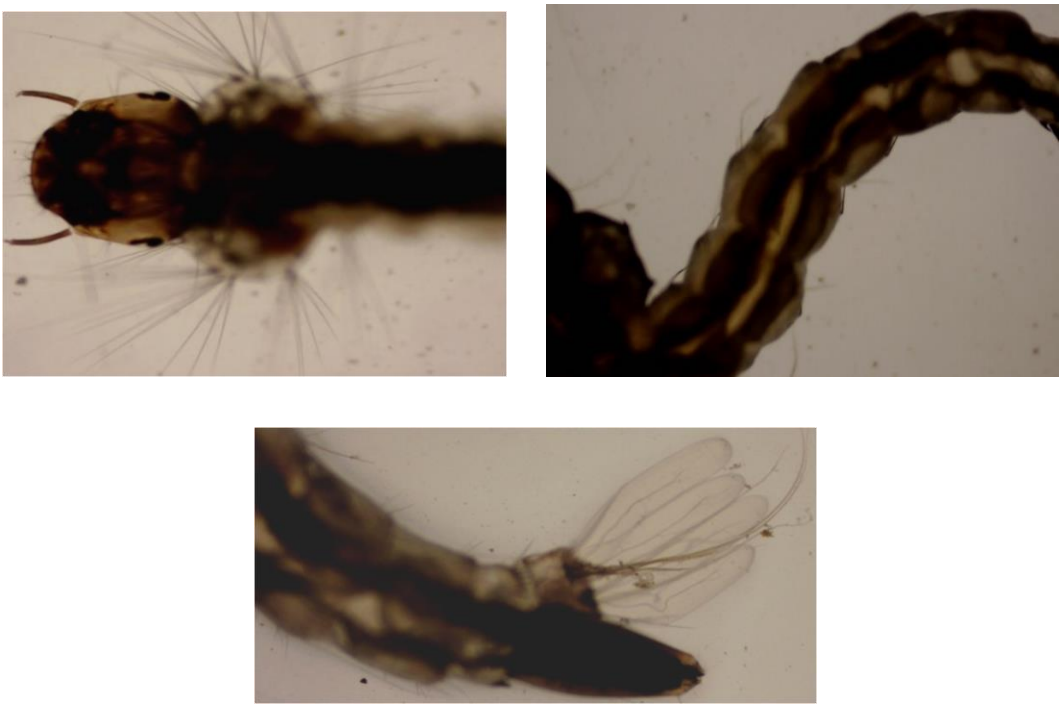
3.3 Preparation of Stock solution

1gram crude extracts dissolved in 100 ml of each of the solvents and stored these as stock solutions. From these stock solutions, desired concentrations of the extracts *viz:* -1mg/ml, 3mg/ml and 5mg/ml were prepared with distilled water.

3.4 Larvicidal activity

Mosquito larvicidal activity was tested against 4th Instar larvae of *Aedes aegypti* L. 10 larvae each were placed in petri dishes containing the following concentrations (1mg/ml, 3mg/ml and 5mg/ml) of the extracts and kept it under observation for 24, 48 and 72 hours respectively. Distilled water was used as a control and mortality percentage was noted as zero in control. The mortality percentage was calculated by using the following formula and the results obtained are presented on **Table No. 2, 3 and 4**.

$$\textbf{Mortality percentage} = \frac{\textbf{No. of larvae died}}{\textbf{No. of larvae introduced}} \times 100$$



(i) Head (ii) Thorax (iii) Abdomen

Plate No: 3 *Aedes aegypti* Linn 4th Instar larvae

4. Results and Discussion

Phytochemical tests and bioactivity of *Vitex negundo* L. leaf extracts against *Aedes aegypti* L mosquito larvae were carried out. Out of the 10 phytochemicals tested 6 phytochemicals were located in aqueous extract and 7 phytochemicals were identified in both acetone and ethanol extracts. The results showed that aqueous extract of *Vitex negundo* L leaves contained carbohydrate, coumarin, flavonoid, phenol, tannin and triterpenoid. Acetone extract possessed alkaloid, coumarin, flavonoid, phenol, tannin and triterpenoid, while ethanol extract showed the presence of alkaloid, carbohydrate, coumarin, flavonoid, phenol, tannin and triterpenoid. Alkaloid was located in both acetone and ethanol extract, while it was absent in aqueous extract. Carbohydrate was identified in aqueous and ethanol extract except aqueous extract. The bioactive principles such as anthocyanin, anthraquinone and protein was absent in all the three extracts tested. The larvicidal activity of aqueous extract showed that on exposure to 24 & 48 hours all larvae exist as alive in all the concentrations, hence mortality percentage was noted as zero. Similar results were recorded on exposure to 24 and 48 hours with 1mg/ml and 3mg/ml. Only 20% mortality was obtained in 5mg/ml on exposure to 72 hours. In 1mg/ml of acetone extract the mortality percentage was 20, 80 and 100% respectively on exposure to 24, 48 and 72 hours. Highest mortality percentage (100%) was noted in 72 hours and lowest (20%) on 24 hours of exposure. Similar results were observed on 3mg/ml and 5mg/ml on different time of exposure (24, 48 and 72 hours) and the mortality percentage was 80 and 100 % respectively. The highest mortality percentage (100%) was noted on 48 and 72 hours and lowest was (80%) on 24 hours of exposure. In ethanol extract the results showed that 1mg/ml concentration the mortality percentage was 10, 90 and 100% respectively. The highest mortality percentage (100%) was noted on exposure to 72 hours and lowest mortality percentage (10%) was on exposure to 24 hours. On exposure to 24 hours at 3mg/ml concentration 40% mortality was noted and in 48 and 72 hours of exposure 100% mortality was obtained. On exposure to 5mg/ml concentration 80% mortality was observed while on exposure to 48 and 72 hours 100% mortality was observed**.** The present study revealed that concentration of the extracts as well as duration of time are the two important factors that leads to the difference in mortality percentage. Larvicidal activity was minimum in aqueous extract while it was maximum in acetone and ethanol extracts due to the presence of alkaloid along with other phytochemicals.

Table 1: Qualitative Phytochemical tests on various extracts of *Vitex negundo* Linn Leaves.

Sl. No	Bioactive principles	Solvents used		
		Aqueous	Acetone	Ethanol
1.	Alkaloid	-	+	+
2.	Anthocyanin	-	-	-
3.	Anthraquinone	-	-	-
4.	Carbohydrate	+	-	+
5.	Coumarin	+	+	+
6.	Flavonoid	+	+	+
7.	Phenol	+	+	+
8.	Protein	-	-	-
9.	Tannin	+	+	+
10	Triterpenoid	+	+	+

"+" Present, " - " Absent

Table. 2: Bioactivity of various concentrations of crude aqueous extract of *Vitex negundo* L leaves.

Sl. No	Larval stage	Concentration of Aqueous extract (mg/ml)	Time (hrs)	No. of larvae introduced	No. of larvae died	Mortality percentage
1	4th Instar larvae	Control	24	10	0	0%
			48	10	0	0%
			72	10	0	0%
2		1	24	10	0	0%
			48	10	0	0%
			72	10	0	0%
3		3	24	10	0	0%
			48	10	0	0%
			72	10	0	0%
4		5	24	10	0	0%
			48	10	0	0%
			72	10	2	20%

Table. 3: Bioactivity of various concentrations of crude acetone extract of *Vitex negundo* L leaves.

Sl. No	Larval stage	Concentration of Acetone extract mg/ml)	Time (hours)	No. of larvae introduced	No. of larvae died	Mortality percentage
1	4th Instar larvae	Control	24	10	0	0%
			48	10	0	0%
			72	10	0	0%
2		1	24	10	2	20%
			48	10	8	80%
			72	10	10	100%
3		3	24	10	8	80%
			48	10	10	100%
			72	10	10	100%
4		5	24	10	8	80%
			48	10	10	100%
			72	10	10	100%

Table. 4: Bioactivity of various concentrations of crude ethanol extract of *Vitex negundo* L. leaves

Sl. No	Larval stage	Concentration of Ethanol extract	Time (hrs)	No. of larvae introduced	No. of larvae died	Mortality percentage
1	4th Instar larvae	Control	24	10	0	0%
			48	10	0	0%
			72	10	0	0%
2		1	24	10	1	10%
			48	10	9	90%
			72	10	10	100%
3		3	24	10	4	40%
			48	10	10	100%
			72	10	10	100%
4		5	24	10	8	80%
			48	10	10	100%
			72	10	10	100%

5. Conclusion

In the present investigation Qualitative phytochemical tests and bioactivity of *Vitex negundo* L. aqueous, acetone and ethanol leaf extracts against *Aedes aegypti* L. larvae were carried out. The results showed that alkaloid was located both in acetone and ethanol extracts while it was absent in aqueous extract. In aqueous extract 20% mortality was obtained only on 5mg/ml concentration on exposure to 72 hours. The effect of aqueous extract is comparatively very low hence less mortality percentage was obtained. In 3mg/ml and 5mg/ml concentrations of both acetone and ethanol extracts, 100% mortality obtained on exposure to 48 and 72 hours respectively. The results revealed that *Vitex negundo* L leaves possessed significant larvicidal activity hence it is used as a natural larvicidal agent.

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