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Research Article

COMPARATIVE LARVICIDAL ACTIVITY OF VARIOUS SOLVENT EXTRACT OF LEAF OF *LIMONIMA ACIDISSIMA* (Linn)

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ABSTRACT

To investigate the Comparative larvicidal activity of various solvent extract of leaf of Limonima acidissima (linn) against the *Culex quinquefasciatus,* Larval mortality was observed after 24 hours of exposure to different concentrations of various solvent extract. From the results, all the extracts exhibit larvicidal activity, however water and ethanol extract showed better results which may be due to the presence of important bioactive compounds in the crude extract.

Keywords: WE- Water Extract , ME- Methanolic Extract, Limonima acidissima (linn), Culex quinquefasciatus,

INTRODUCTION

Repeated use of synthetic insecticides for mosquito control has resulted in the development of resistance [1] undesirable effects on non-target organisms and fostered environmental and human health concern [2] which initiated a search for alternative control measures. Plants are considered as rich sources of bioactive chemicals [3, 4] which may be alternative sources of mosquito control agents. Phytochemicals derived from plant sources can act as larvicide,insect growth regulators, repellent and oviposition attractant and have different activities observed by many researchers[5, 6] The leaf extracts of several plant species showed encouraging results against *Aedes aegypti* (Ae. aegypti)[7,8] *Culex quinquefasciatus* (Diptera: Culicidae) (Cx. *quinquefasciatus*) [9] and *Anopheles stephensi* (An. stephensi) mosquito larvae[10] *Limonia acidissima L*. (Rutaceae) (*L.*

acidissima) the Indian wood apple is a multistemed tree, distributed in tropical and temperate regions of the world and is a large tree growing to 9 m tall, with rough, spiny bark. The leaves are pinnate, with 5-7 leaflets, each leaflet 25-35 mm long and 10-20 mm broad, with a citrus-scent when crushed. The fruit is a berry, 5-9 cm in diameter, and may be sweet or sour. The unripe fruit acts as astringent and is used in combination with bael and other medicines, in diarrhoea and dysentery. The fruit is much used in India as a liver and cardiac tonic, and when unripe, as an astringent means of halting diarrhoea and dysentery and effective treatment for hiccough, sore throat and in the diseases of the gums. The ripe fruit is rich in carotene, a precursor of Vitamin A; it also contains significant quantities of the B vitamins, thiamine and riboflavin, and small amounts of Vitamin C. The root juice was once popular as a remedy for snakebites. The seed oil is a purgative, and the leaf juice mixed with honey is a folk remedy for fever. The tannin-rich and alkaloid-rich bark decoction is a folk cure for malaria [11, 14] . The objective of the present study is to examine the role of leaf extract of *L. acidissima* as a biocontrol agent against the larval forms of *Cx. quinquefasciatus* and its characterization

MATERIAL AND METHODS

Collection of Plant Material

The plant material *Limonia acidissima* was collected from Alangulam in virudhunagar District and taxonomically identified with help of Dr.S.David Noel, Assistant Professor Department of Botany Sri Kaliswari College, Sivakasi (Autonomous) and the voucher specimen is stored in department Herbarium for further reference.

Preparation of Extract [15-17]

The collected plant materials were initially rinsed with distilled water to remove soil and other contaminants. The leaves of *L. acidissima* were separated and dried at 37°C for 14 days. The dried leaves were powdered and stored in plastic bags for the next step. A 20 gm of sample powder was extracted using 100% of water, 100 % of ethanol, 50% of water & ethanol, 100%methanol has been dipped for 3days prior to inhibition study. Then it was shaken for 6 hrs (per day 3 times shaken well) and filtered using what man NO: 1 filter paper. It has to be boiled

at 80 ºC for 30 minutes. Then all the extract was evaporated using hot air oven to produce a dry powder. The final dry plant powder was dissolved in solvents and used for further work.

Phytochemical screening[5-17]

All the four extract were subjected to preliminary phytochemical screening tests using various reagents.

Larvicidal Activity[18, 19]

The larvae of *Culex quinquefasciatus* was collected from ICMR (Indian Council Medical Research) Madurai and used for this study. 25 larvae were taken at a two different 500ml glass beakers.

1ml of water extract was added to one beaker. It was considered as a control. 1ml of acetone was mixed with another beaker containing larvae. Then these are incubated for 24hrs at room temperature.

After 24hrs the mortality rate was recorded and assessed[18]. This study was done in triplicates and further statistical analysis was done. The mortality percentage was calculated (Abbortt's 1925)

Percentage mortality of larvae = No. Of dead larvae / No. of larvae introduced X 100

Abbortt's Formula

P = PI-C/1-C

Where, PI and C denote the observed mortality and the natural mortality.

RESULTS AND DISCUSSION

The activity of crude plant extracts is often attributed to the complex mixture of active compounds. Phtyochemical screenings reveals mixed results. Aqueous extract attributed maximum positive results for the tested compounds Alkaloid, flavanoid, Phytosterols proteins etc., (Table-1).

Previous workers reported that fruit pulp contains large quantity of citric acid and other fruit acids, mucilage and minerals. Alkaloids, coumarins, fatty acids and sterols have been detected in the pericarp.

It also contains umbelliferone, dictamnine, xanthotoxol, scoparone, xanthotoxin, isopimpinellin, isoimperatorin and marmin[31]

Table 1: Preliminary Phytochemical Screening

S. No	Test For	Reagent Used	100 % Water	100 % Ethanol	50 %Water + Ethanol	100% Methanol
I	Alkaloid	1.Wagner's test	+++	+	++	++
		2.Hager's test	++	+	+++	++
		3.Mayer's test	+	++	+	++
		4.Picric acid test	+++	+	++	+++
2	Flavanoid Test	1.Alkaline Reagent	++	_	+	+
		2.Lead acetate test	+++	+	++	+
		3.Ammonia test	+	++	+	+
3	CHO test	1.Benedicts reagent	+++	_	+	+
		2.Fehling's reagent	++	_	+++	++
		3.Conc.H_2SO_4 test	+++	_	++	+
4	Proteins & amino acids	1.Xanthoproteic test	-	++	+	_
		2.Ninhydrin test	_	_	_	+
		3.Biuret test	+++	++	+++	+
5.	Glycosides Test	1.Modified Borntrager's test	+	_	+	_
		2.Keller killiani test	++	+	+++	++
	Steroids and	1.salkowski's test	+	_	+	+

6.	Terpenoids Test	2. Libermann Burchard test	++	++	_	+
7.	Inorganic compound test	1.sulphate test	+++	+	++	++
		2.carbonate test	–	+++	++	+
8.	Saponins	1.Foam test	+++	++	_	++
		2.Froth test	++	+	_	+
9.	Anthra-Quinones	Borntrage's Test	+	–	–	–
10.	Tannins & Phenols	1.$FeCl_3$ test	++	+	+++	++
		2.Gelatin test	++	_	+	_
		3.Lead –sub acetate test	++	_	+	+++
11.	Resins	Acetone test	–	–	–	–
12.	Organic acid	1.Oxalic acid	+++	+	++	++
		2.Mallic acid	+++	+	++	++
13.	Gum And Mucilage	Ppt By Alcohol	–	–	+	+
14.	Fixed Oils And Fats	Spot Test	++	_	+	+

+++ *Maximum concentration /presence of phytochemical*

- - - *lower concentration /absence of phytochemical*

\- *Nil*

LARVICIDAL ACTIVITY

Table. 2 Larvicidal toxicity effect of *Limonia acidissima* (L) leaf of water extract of *Culex quinque fasciatus*.

S.NO	Extract	LARVAL STAGE	DOSAGE (mg)	LARVAL MORTALITY CONCENTRATION (ppm)	LC 50 VALUE (%)
1	WE	1	20	6	44.40
2	WE	2	40	2	48.98
3	WE	3	60	5	45.12
4	WE	4	80	5	45.72
5	WE	5	100	2	48.00
6	EtOH	1	20	13	37.40
7	EtOH	2	40	8	42.10
8	EtOH	3	60	6	44.48
9	EtOH	4	80	4	46.73
10	EtOH	5	100	2	48.12
11	50%EtOH	1	20	20	30.15
12	50%EtOH	2	40	16	34.69
13	50%EtOH	3	60	10	40.12
14	50%EtOH	4	80	6	44.79
15	50%EtOH	5	100	4	46.80
16	ME	1	20	17	33.10
17	ME	2	40	14	36.80
18	ME	3	60	11	39.90
19	ME	4	80	7	43.40
20	ME	5	100	6	44.44

From the results, all the extracts exhibit moderate larvicidal activity after 24 hours exposure in dose dependent manner, However water and Ethanolic extract showed more or less similar activity at 2 ppm concentration where LC50 % is 48.12 % at 100 mg (Table-2) comparing to

other tested extracts may be due to the presence of important bioactive compounds the preliminary phytochemical studies strengthens our finding

In the present study *L. acidissima* leaf extract produced mortality against the target mosquito species which might be due to the actions of a particular bioactive compound or synergistic effects of others. Phytochemical analysis of the leaf extract revealed the presence of natural botanicals are playing an important role as a suitable alternative to synthetic pesticides, whose application is safe due to vast availability and their easy degradable property [18].

Although several plants from different families have been reported for mosquitocidal property [20, 21] only a few botanicals have moved from laboratory to field use like *Chrysanthemum cinerarifolium* (Family: Compositae) [22] which has also been used in indoor sprays [23]. Different types of biological activities are played by a wide variety of secondary metabolites of plants. Most studies reported active compounds responsible for mosquito larvicidal property as steroidal saponins. Wiesman & Chapagain [24] revealed that saponin extracted from the fruit of *Balanites aegyptica* showed 100% mortality against larvae of *Stegomyia aegypti* (*S. aegypti*).

The larvicidal property of a saponin mixture isolated from *Cestrum diurnum* was also evaluated against An. stephensi mosquito by Ghosh & Chandra [10]. Alkaloids derived from *Piper longum* fruit and *Triphyophyllum pellatum* reported by Lee [25] and Francois *et al.*[26] exhibited larvicidal activity against *Culex pipiens* (*Cx. pipiens*) and *An. stephensi*, respectively.

Joseph *et al*.[27] showed isoflavonoids from tubers of *Neorautanenia mitis* had a larvicidal effect against the mosquitoes, *Anopheles gambiae* and *Cx. quinquefaciatus*, respectively. The impact of phenolic compounds on the mosquito larvae has also been evaluated [28- 30]

Previous workers also reported that Acetone extract of the dried leaves of *L. acidissima* found to be effective against larvae of *Culexquinque fasciatus*, *Anopheles stephensi* and *Aedes aegypti*, with LC50 of 129.24, 79.58 and 57.23 ppm, respectively (Abdul *et al*., 2000)

Leaf extract of *Limonia acidissima* L. (Rutaceae) as a biocontrol agent against the larval form of *Culex quinquefasciatus*, and characterization of bioactive component responsible for larvicidal activity (18). Larval mortality of mosquito species was observed after 24, 48 and 72 hours of exposure to different concentration of aqueous extract, solvent and subsequently bioactive compound . act as larvicide.

Acetone extract of the dried leaves found to be effective against larvae of *Culexquinque fasciatus, Anopheles stephensi* and *Aedes aegypti*, with LC50 of 129.24, 79.58 and 57.23 ppm, respectively [(19)]

CONCLUSION

Our findings showed that water and Ethanol extract of *L. acidissima* leaves showed larvicidal activity against *Culex quinquefasciatus.* Further phytochemical elucidation studies needed to put the drug into therapeutic use. Also our results open the possibility for further investigations of the efficacy of larvicidal properties of natural product extracts.

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