ISSN PRINT 2319 1775 Online 2320 7876

Journal Volume 10,Iss 04, 2021

PHYTOCHEMICAL SCREENING AND LARVICIDAL ACTIVITY STUDY ON *CLERODENDRUM PHLOMIDIS* L.F. AGAINST *AEDES AEGYPTI* (LINN.) LARVAE

J.V. Jincy[1] (Reg. No.: 20113162262021)
Ph. D. Scholar (Full- Time)
Department of Botany and Research Centre,
Scott Christian College (Autonomous), Nagercoil – 629003,
(Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli – 627012),
Tamil Nadu, India.

S. Thampiraj[2]
Associate Professor,
Department of Botany and Research Centre,
Scott Christian College (Autonomous), Nagercoil – 629003,
(Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli – 627012),
Tamil Nadu, India.

[1]Corresponding author e-mail: jincyjv222@gmail.com

Abstract

This study was undertaken to assess the phytochemical and pesticidal activities of *Clerodendrum phlomidis* stem, leaf, root and flower extracts. Bioactive compounds were extracted using acetone, benzene, ethanol n- butyl alcohol and isopropyl alcohol for phytochemicals investigation. Most of the extract revealed the presence of alkaloid, flavonoid, phenol, tannin and saponin, carbohydrate, glycosides, proteins, terpenoids**.** Plants may form an alternative sources of mosquito control agents. The present study also assessed the larvicidal activity of stem, root, leaves and flower of *Clerodendrum phlomidis* L.F. plant parts collected, dried, powdered then extracted using acetone, benzene, ethanol n- butyl alcohol and isopropyl alcohol. Larvicidal activity conducted by serial dilution methods in different concentrations such as 100, 200, 300,400 and 500 ppm. The larval mortality was calculated after 12 h and 24h of the exposure period. LC_{50} and LC_{90} values were calculated by probit analysis. All plant extracts showed significant larvicidal activity against *A. aegypti* mosquito larvae. Among the extract tested, the n-butyl alcohol extract recorded the highest activity in all the extracts. In comparing plant parts, root shows more activity than other parts. Root of n- butyl alcohol extract attaining 87% mortality against *Aedes aegypti* after 24h of exposure period. Present study reveals that plant extracts as biocidal agent against mosquito larvae. Our data suggest that the stem, root, leaves,

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flowers of n-butyl alcohol extract of *Clerodendrum phlomidis* have the potential to be used as an eco-friendly approach for the control of the *Aedes aegypti.*

Keywords: Larvicidal activity, *Clerodendrum phlomidis*, *Aedes aegypti.*

Introduction

Clerodendrum phlomidis is important and well-known medicinal plant one of the highly traded plants from tropical forest as the leaves and root are widely used in unani, siddha, ayurveda and folk medicine to treat various disease (chellaih muthu *et al.*, 2012). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plant produce these chemicals to protect themselves but recent research demonstrate that they can also protect humans against diseases (Abd Egadir *et al.*, 2014).

Mosquitoes serve as vector for various tropical and subtropical diseases which cause destructive effects to human. They do not only transmit parasites and pathogens but they also source of allergic reaction that includes local skin and systemic sensitivity (Cheng *et al.*, 2003). The most common diseases associated with mosquitoes are dengue fever, chikungunya, yellow fever and the worst, dengue haemorrhagic fever where Aedes aegypti is one of the mosquito species responsible for the transmission of these vector borne diseases.

The technique in controlling mosquitoes depends on the larval stages (egg, larvae, pupae, and adult) on target. Mosquito control includes targeting the adult mosquito through spraying chemical insecticides or by killing the mosquito larvae before they emerge into adults via using synthetic larvicides or botanical extracts as an alternative larvicide (Tiwary *et al.*, 2007). However, the use of synthetic larvicides imposes threats not only to human health but also to the ecosystem because when they are applied into the environment; they may stay on for a very long time or even remain there without end. (Mathivanan *et al*., 1987). Synthetic larvicides also disrupt natural biological control systems that sometimes results into a widespread development of resistance. (Mathivanan *et al.*, 2000). This phenomenon has triggered and urged the development of alternative techniques using natural products. Instead of using synthetic larvicides, the use of these plant-derived products in controlling mosquito larvae is inexpensive and environment-friendly (Arnason *et al*., 1989)

Materials and methods:

Plant material

Healthy stem, root, leaves and flowers of *Clerodendrum phlomidis* collected from Kalkulam taluk of Tamil Nadu, India. The plant materials were cleaned, air-dried at room temperature for two weeks and coarsely powdered.

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Preparation of extracts

Powdered plant materials were extracted successively by using different solvents of acetone, benzene, ethanol, n- butyl alcohol and isopropyl alcohol extracts in Soxhlet apparatus for 18 h and the extractives were filtered through Whatsman filter paper then these extracts were concentrated and kept in brown bottles used for the further study.

Phytochemical analysis

The extracts were tested for alkaloid (Edeoga *et al.*,2005), flavonoid (Boham *et* Kocipai-Abyazan, 1994), phenol (Sadasivam *et* Manickam, 1992), tannin (Van – Burden *et* Robinson, 1981) and saponin (Obadoni *et* Ochuko, 2001),carbohydrate, glycosides,proteins,terpenoids.

Mosquito Larvicidal Bioassay

The eggs of *Aedes agyptii* were obtained from CRME (Centre for Research in Medical Entomology), Madurai. The larvae were fed Brewer's yeast: dog biscuit (1:3). The larvae at fourth instar stage were used for larvicidal assay. The efficacy of the plant extracts as larvicide against the dengue-vector *Aedes aegypti* mosquito was evaluated in accordance with the guidelines of World Health Organization 2003. Batches of 20 third-instars larvae of *Aedes aegypti* were placed in Laboratory room at 30-32^{0}C. For the control group, the mosquito larvae were exposed to 60 mg/mL of chloroform, ethyl acetate, benzene, n-butyl alcohol, ethanol, petroleum ether, and aqueous solvents since it is the solvent used in the extraction of different plant samples. The experimental group is the extracts of acetone, benzene, ethanol, n- butyl alcohol and isopropyl alcohol the stem, root, leaves and flowers of *Clerodendrum phlomidis* with 200, 400, 600, 800 and 1000 ppm concentrations. Each treatment was conducted in three replicates. The effects of the plant extracts were monitored through carefully counting the number of dead larvae after 12and 24 hours of treatment, and the percentage mortality was computed.

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

Statistical Analysis:

The statistical tools that were used in this study are the following: the Arithmetic Mean to get the average number of dead of mosquito larvae, Analysis of Variance (ANOVA), to determine the significant difference on the mortality of mosquito larvae between the control and the experimental groups, Scheffe Test to test the degree of variability between the control and different concentrations of the plant samples, and Probit Analysis to calculate LC_{50} and LC_{90} values to determine Lethal concentrations of the plant extracts on *Aedes aegypti* mosquito larvae after 12 and 24 hours of treatment.

Result and discussion

Phytochemical analysis was done to analyse the chemical constituents present in the acetone, benzene, ethanol n- butyl alcohol and isopropyl alcohol for phytochemicals investigation. Most of the extract revealed the presence of alkaloid, flavonoid, phenol, tannin and saponin. (Table 1).

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ethanol extract shows highest amount of bioactive compound than other solvent extracts in all plant part. protein, phenol, steroids are present in all solvent and all parts. Flavonoids, glycosides, saponin were absent in all extract and all parts of plant. In stem ethanol extract shows high amount of bioactive compound. ethanol extract shows alkaloids, proteins steroids, phenol, tannin and terpenoids. acetone shows low amount bioactive compounds, this shows carbohydrate, proteins, phenol and terpenoid. Yogesh 2015 conducted Phytochemical investigation of the plant *Clerodendrum phlomidis* (Lamiaceae). The isolate of two new flavonoid glycosides (1, 2) together with six known compounds identified as pectolinaringenin (3), pectolinaringenin-7-O-β-D-glucopyranoside (4), 24β-ethylcholesta-5,22E,25-triene-3β-ol (5), 24β-ethylcholesta-5,22E,25-triene-3β-O-β-D-glucopyranoside (6), (2S,3S,4R,10E)-2-[(2'R)-2'-hydroxytetracosanoylamino]-10-octadecene-1,3,4-triol (7) and andrographolide (8) mainly by spectroscopic analysis. Yatheesharadhya et al., 2021 reported that the leaves of *C. phlomidis* revealed presence of biological active molecules like carbohydrates, saponins, alkaloids, flavonoids, phenolics and tannins, phytosterols and triterpenoid. The activity exhibited in this study might be due to the presence of these phytochemicals in the species investigated.

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Solvent extracts	samples	Alkaloid	Carbohydrate	flavonoids	glycosides	Proteins	Saponins	Steroids	Phenol	Tannin	Terpenoid
Acetone	Stem	+	+	-	+	+	-	+	+	_	+
	Root	+	+	-	+	+	-	+	+		-
	Leaf	+	+	-	-	+	-	+	+	+	+
	Flower	+	+	-	-	+	-	+	+	-	_
Benzene	Stem	+	+	-	+	+	-	+	+	+	+
	Root	+	+	-	+	+	-	+	+	-	-
	Leaf	+	+	-	-	+	-	+	+	-	-
	Flower	+	+	-	-	+	-	+	+	+	-
Ethanol	Stem	+	+	-	+	+	-	+	+	+	+
	Root	+	+	-	+	+	-	+	+	+	+
	Leaf	+	+	-	-	+	-	+	+	+	+
	Flower	+	+	-	-	+	-	+	+	-	_

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N-Butanol	Stem	+	+	-	+	+	-	+	+	+	+
	Root	+	+	-	+	+	-	+	+	-	+
	Leaf	+	+	-	-	+	-	+	+	-	+
	Flower	+	+	-	-	+	-	+	+	-	+
Isopropyl alcohol	Stem	+	+	-	+	+	-	+	+	+	-
	Root	+	+	-	+	+	-	+	+	+	-
	Leaf	+	+	-	-	+	-	+	+	+	-
	flower	+	+	-	-	+	-	+	+	-	+

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Larvicidal activity of different solvent extracts of *Clerodendrum phlomidis* stem, root, leaf, flower was studied against *Aedes aegypti*. The results revealed that all the extracts showed various level of larvicidal activity against *Aedes aegypti* after 12h and 24h of the exposure periods. Among the extract tested, the n – butyl alcohol extracts recorded the highest larvicidal activity.

Larvicidal activity of *Clerodendrum phlomidis* stem extract

Among the extract tested the highest mortality was observed in n- butyl alcohol extract with the LC_{50} and LC_{90} values 417.7543 and 367.0196 ppm after 12h and 367.0196 and 637.425 ppm after 24 h. followed by ethanol extract after 12h they showed 412.0578 and768.4256.24h showed 383.4284 and ppm. lowest mortality rate showed benzene extract was 891.9509 and 967.78 ppm after 12h and 831.994 and 902.667 ppm after 24h. increasing concentration percentage of mortality increases. N- butyl alcohol extract of Clerodendrum phlomidis stem possesses good mortality activity than others. After12h, they reach 73% mortality, after 24h they reach 83%.

Fig1. LC_{50} and LC_{90} values of n-butyl alcohol extract in stem

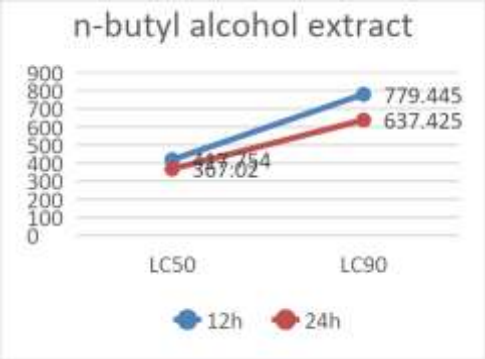
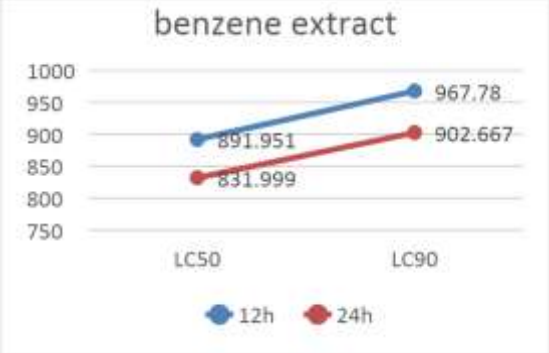


Fig 2 LC_{50} and LC_{90} values of benzene extract in stem



Larvicidal activity of *Clerodendrum phlomidis* root extract

Among the extract tested the highest mortality was observed in n- butyl alcohol extract with the LC_{50} and LC_{90} values 411.3989 and 740.2553 ppm after 12h and 365.3153 and 645.263 ppm after 24 h. followed by ethanol, isopropyl, chloroform extract lowest mortality rate was showed benzene extract 1047.9167 and 1098.484 ppm after 12h and 875.4863 and 987.332 ppm after 24h. N- butyl alcohol extract of *Clerodendrum phlomidis* root possesses good mortality activity than others. After12h, they reach 79% mortality, after 24h they reach 87%.

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Fig 3. LC_{50} and LC_{90} values of n-butyl alcohol extract in root

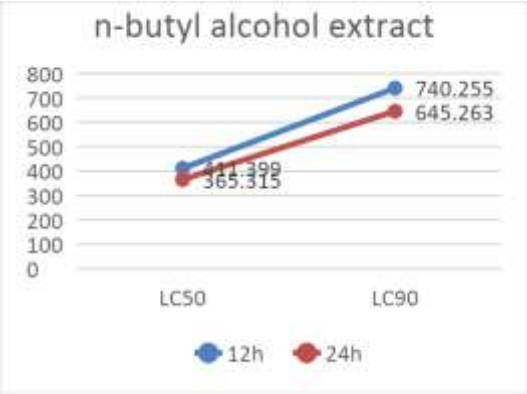
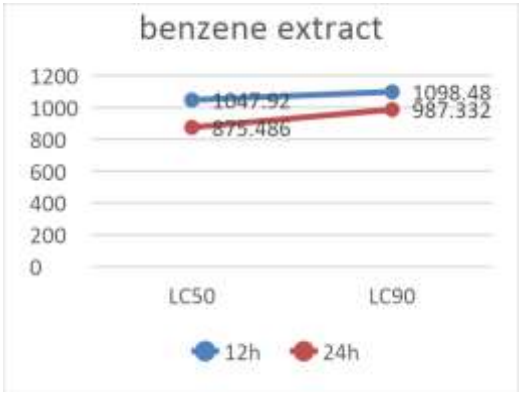


Fig 4. LC_{50} and LC_{90} values of benzene extract in root



Larvicidal activity of *Clerodendrum phlomidis* leaf extract

Among the extract tested the highest mortality was observed in n- butyl alcohol extract with the LC_{50} and LC_{90} values 374.3757 and 657.123 ppm after 12h and 368.373 and 645.678 ppm after 24 h. lowest mortality rate was showed benzene extract 1501.6688 and 1678.345 ppm after 12h and 937.4921 and 1189.456 ppm after 24h. N- butyl alcohol extract of *Clerodendrum phlomidis* leaf possesses good mortality activity than others. After12h, they reach 74% mortality, after 24h they reach 84%.

Fig 5. LC_{50} and LC_{90} values of n-butyl alcohol extract in leaf

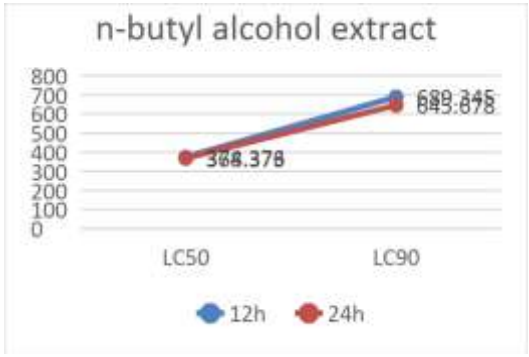
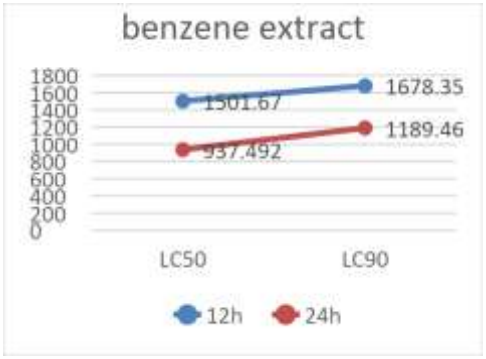


Fig 6. LC_{50} and LC_{90} values of benzene extract in leaf



Larvicidal activity of *Clerodendrum phlomidis* flower extract

Among the extract tested the highest mortality was observed in n- butyl alcohol extract with the LC_{50} and LC_{90} values 395.5581 and 593.567 ppm after 12h and 374.0414 and 567.455ppm after 24 h. followed by ethanol extract, isopropyl alcohol, lowest mortality rate was showed by benzene extract. They showed 942.9023 and 1067.45 ppm after 12h and 374.0414 and

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567.455 ppm after 24h. N- butyl alcohol extract of *Clerodendrum phlomidis* flower possesses good mortality activity than others. After12h, they reach 75% mortality, after 24h they reach 85%.

Fig7. LC_{50} and LC_{90} values of n-butyl alcohol extract in flower

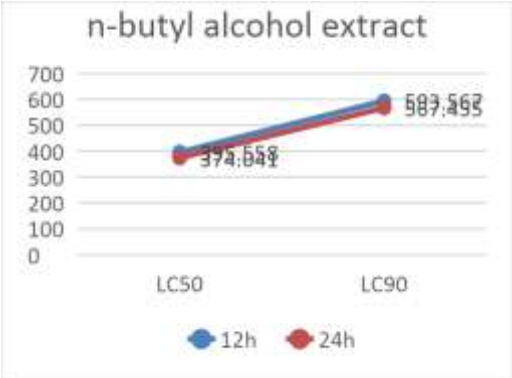
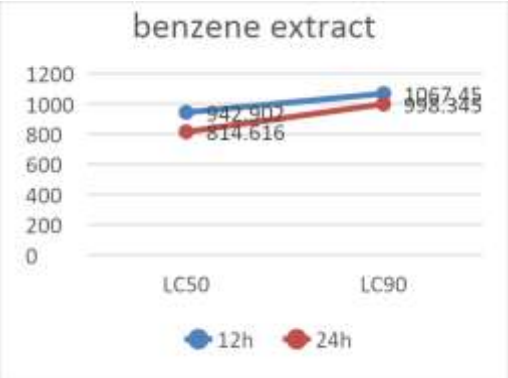


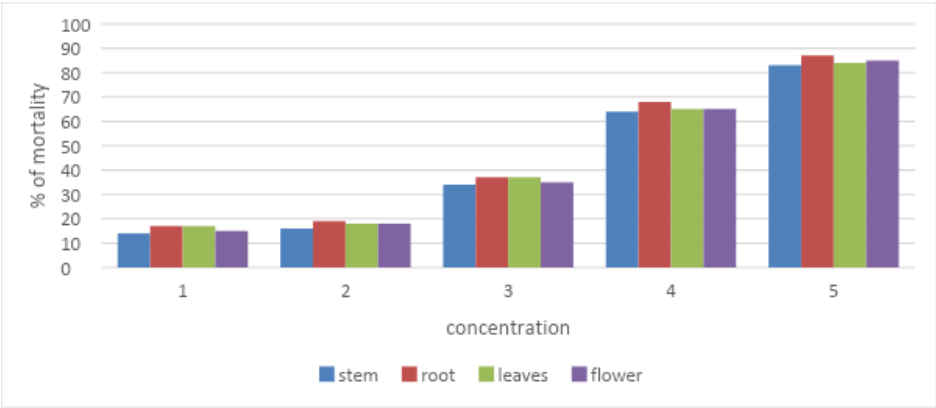
Fig 8. LC_{50} and LC_{90} values of benzene extract in flower



Comparison of larvicidal activity with different parts of *Clerodendrum phlomidis*

All parts of *Clerodendrum phlomidis* shows good larvicidal activity with different solvent extract. Comparing larvicidal activity with different parts revealed that root shows more activity than others in the term of L_{50} and LC_{90} values and % of mortality. Followed by leaf, flower and stem.

Fig 9.Comparison of percentage of mortality in different parts after 24h



In the present investigation, the mortality percentage was increasing with the increasing concentration and increasing time intervals in all extract. Similarly increased rate of mortality with an increasing time was observed by promsiri *et al.*, (2006). Similar trend was noticed by many researchers Ramar and Jeyasankar (2014). Jayasankar *et al.*, 2015 demonstrates the

ISSN PRINT 2319 1775 Online 2320 7876

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efficacy of leaf extract of *Clerodendrum phlomidis* as an effective larvicide against larvae of *Aedes aegypti*, *An. stephensi* and *Cx. quinquefasciatus.*

Conclusion:

Present study reveals that *Clerodendrum phlomidis* possess large amount of phytochemicals. These phytconstituents play major role in the medicinal field. Plant extracts act as biocidal agent against mosquito larvae. On comparing with different parts, root shows more activity than other parts. The mortality of mosquito larvae increases with increasing concentrations of the plant extracts and time intervals. n- butyl alcohol extract shows most powerful efficacy in all plant parts. *Clerodendrum phlomidis* extract can play an important role in the management of the mosquitoes. Besides this natural product has no side effect in agriculture and human health. Through this study it was proved that n- butyl alcohol of *Clerodendrum phlomidis* extracts were considered the best in terms of LC_{50} values, LC_{90} values as well as in terms of percent mortalities as compared to other extracts. Hence, the study authenticate the utility of the investigated specieseffectively in mosquito managing programmes.

Acknowledgement:

The authors are thankful to the Principal, Head of the Department of Botany and Management of Scott Christian college (Autonomous), Nagercoil for providing laboratory facilities during the period of this study.

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