International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Screening of *Lawsonia inermis* (Lythraceae) Leaf Extract for its Ovicidal Efficacy Against the Mosquito *Culex quinquefasciatus* (Diptera:Culicidae)

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Abstract: ***Mosquitoes are the most important vectors of certain human infectious diseases. The control of mosquito-borne diseases is however becoming increasingly difficult because the effectiveness of vector control has declined due to development of resistance by vectors against the currently used but toxic and environmentally persistent insecticides. Naturally-occurring phytocompounds that are rich sources of bioactive chemicals appear to be the most likely candidates for the environmentally safe, degradable and target specific against mosquitoes. Therefore the present study was designed specifically to investigate the mosquito ovicidal activity of different solvent extracts of Lawsonia inermis against selected mosquito Culex quinquefaciatus. The ovicidal activity was determined at concentrations of 100, 150, 200, 250 and 300 ppm and hatchability was assessed after 48 hours. Highest percentage of ovicidal activity was recorded in petroleum ether extract. The qualitative phytochemical analysis of the leaf extract revealed the presence of alkaloids, tannins, terpenoids, sterol, saponins, anthraquinones, proteins and quinones.. These result suggested that the leaf extracts of Lawsonia inermis have the potential to be used as an ideal eco friendly approach for the control of the Culex quinquefaciatus.***

Keywords: Ovicidal, *Lawsonia inermis*, *Culex quinquefaciatus*, Mosquito, Plant extracts, Pytocompounds

1. Introduction

Mosquitoes are found throughout the world excluding the places which are completely frozen. Three fourth of the existing mosquito species are native to tropical and subtropical region. They are likely to transmit disease to more than two fifth of the world population (Navaneethan *et al*., 2016). They are the vectors for the dreadful diseases of mankind. Of all the insects that transmit diseases, mosquitoes represent the greatest menace. WHO has declared the mosquito “public enemy number one” because they are responsible for the transmission of various dreadful disease causing pathogens (WHO, 1996). It spreads many diseases such as filariasis, malaria, dengue, yellow fever and Japanese encephalitis which contribute significantly to disease burden, death, poverty and social debility in tropical countries (Jang *et al.,* 2002).

The mosquito *Culex quinquefasciatus* acts as a vector for *Wuchereria bancrofti* responsible for filariasis. It is widely distributed in tropical and subtropical countries with around 120 million people infected worldwide and 40 million people having common chronic manifestation (Berhard and Berhard, 2003). There are 45 million cases of lymphatic filariasis in India alone (Bowers *et al*., 1995). Estimates suggest that about 120 million people over 73 countries are infected with human lymphatic filariasis (Southgate, 1984 and WHO, 1997).

Mosquito control is at present the only way to limit these vector borne diseases (Mariappan, 2007). One of the measures for mosquito control is the use of chemical insecticides. It is favourable because of their speedy action and easy application. The relative toxicity of insecticides to various mosquito species has been studied by entomologist in detail (Rajavel *et al.*, 1987; Saxena and Koushik, 1988). Synthetic insecticides are toxic and adversely affect the environment by contaminating soil, water and air. Chemical control of mosquitoes is also causing many unwanted effects on human health and non target animals (Babu and Murugan, 1988). The increased use of these insecticides may enter into the food chain. They even result in mutation of genes and these changes become prominent only after a few generations (Ghose, 1991). The present scenario for commanding the mosquitoes is aimed at application of target and stage specific, cost effect and bio degradable phytoproducts (Murugesan *et al.,* 2015).

Control of vector mosquitoes is relentlessly carried out to reduce mosquito borne disease burden in many countries in the world. Various tools and strategies targeting immature and adult mosquitoes are employed for effective control. There are, however, no tools available for large scale control of vector mosquitoes at the embryonic stage. In recent years, more attention has been given to screen plants for their phytocompounds that can cause disruption in development of embryo in eggs laid by mosquitoes. Many researchers have reported plant extracts to possess ovicidal activity against mosquitoes (Govindarajan, 2011 a,b; Govindarajan *et al*., 2011a, 2012, 2013; Tennyson *et al*., 2011; Krishnappa and Elumalai, 2012; Krishnappa *et al*., 2013; Kovendan *et al*., 2013).

Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. The phytochemical analysis of the medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases (Wadood *et al*., 2013). Botanical pesticides have been used traditionally by

human communities in many parts of the world against insect pest (Jacobson, 1958). Natural products are preferred because of their innate biodegradability (Ananthakrishnan, 1988). Biologically active materials derived from plant sources which can act as larvicides, insect growth regulators, repellents, oviposition attractants and deterrents are observed by many researchers (Venkatachalam and Jebanesan, 2001a, b). Green plants are the store houses of many chemical components. In recent years the popularity of complementary medicine has increased. Over 50% of modern drugs are natural products (Joshi, 2000; The Wealth of India, 2003; Khare, 2011) and they play an important role in drug development program of pharmaceutical industry (Baker *et al*., 1995).

Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols, flavonoids etc, which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, root, etc. The beneficial medicinal effects of plant materials typically result from the combination of these secondary products (Tonthubthimthong *et al*., 2001). The medicinal plants are useful for healing as well as for curing of human disease because of the presence of phytochemical constituent (Nostro *et al.,* 2000). The plant has been shown to have potential in treating a number of ailments where the free radicals have been reported to be the major factors contributing to the disorders. However, no information was available on the ovicidal activities of the plant species *L. Inermis* against *C. quinquefasciatus*. Therefore, the aim of this study was to investigate the mosquito ovicidal activities of the different solvent extracts of the selected plant.

2. Materials and Methods

2.1 Origin and maintenance of the mosquito colonies

Mosquitoes used in study were *Culex quinquefasciatus*. Individuals were reared for several generations in the Department of Zoology, Nirmala College for Women, Coimbatore by Hay infusion method under laboratory conditions.

2.2 Collection of leaves and preparation of leaf powder

Fully developed fresh leaves of the plant *L. inermis* were collected from natural habitat of Coimbatore locale, Tamil Nadu, India. They were, washed in water and dried under shade at room temperature for 2 to 3 weeks and were powdered using an electric pulverizer. Fine powder was obtained by sieving.

2.3 Preparation of extracts

10 g the leaf powders was weighed using an electronic balance and were subjected to extraction (Harbourne, 1973 and Vogel, 1978). Petroleum ether extraction was followed by chloroform and ethanol extraction in their increasing order of polarity. The leaf extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath. The residue thus obtained was used for further bioassays.

2.4 Ovicidal Bioassay

Ovicidal activity was assessed by the slightly modified method of Su and Mulla, 1998. The egg raft/eggs of *C. quinquefasciatus* were collected from Department of Zoology, Nirmala College for Women, Coimbatore. The *L. inermis* leaf extracts were diluted in the appropriate solvents to achieve various concentrations ranging from 100 to 300 ppm. Eggs of the mosquito species (100 nos.) were exposed to each concentration of *L. inermis* leaf extracts. After treatment the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope.

Each experiment was replicated three times along with appropriate control. The hatch rates were assessed 48 h after treatment and counts were made every 24 h after exposure until the test was terminated. The hatch rates were assessed by the following formula.

$$\%\ \text{of egg mortality} = \frac{\text{Mortality at treatment} - \text{Mortality at control}}{100 - \text{Mortality at control}} \times 100$$

Statistical analysis

The data on bioassay studies were also subjected to One Way Analysis of Variance (ANOVA) as described by Panse and Sukahtme (1985). The egg mortality data were subjected to probit analysis (Finney, 1971).

Phytochemical screening

Qualitative analysis

Preliminary phytochemical screening of leaf extract of selected plant was carried out using the standard procedures.

Test for Alkaloids

- **Mayer's test (Evans, 1997):** A fraction of extract was treated with a drop or two of Mayer's test reagent along the sides of test tube and observed for the formation of white or cream coloured precipitate.
- **Wagner's test (Wagner, 1993):** A fraction of extract was treated with Wagner's reagent along the sides of the test tube and observed for the formation of reddish brown colour precipitate.
- **Hager's test (Waget *et al*., 1996):** A few ml of extract was treated with 1 or 2 ml of Hager's reagent and observed for the formation of prominent yellow precipitate.

Test for Tannins

- **Ferric chloride test (Trease and Evans, 2002):** About 0.5 g extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate, and observed for the blue-black, green or blue-green precipitate

Test for Phenols

- **Ferric chloride test (Mace, 1963):** The extract (50mg) was dissolved in 5 ml of distilled water and treated with few drops of 5% ferric chloride and observed for the formation of dark green colour

- **Lead acetate test (Brain and Turner, 1975 and Evans, 1996):** The extract (50 mg) was dissolved in 5 ml of distilled water and 3 ml of 10% lead acetate solution was added and observed for the formation of bulky white precipitate.

Test for Flavonoids

- **NaOH test (Trease and Evans, 2002):** Few quantity of the extract was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.
- **Lead acetate test (Brain and Turner, 1975 and Evans, 1996):** Test extract (50 mg) was taken in a test tube and few drops of lead acetate solution was added to it and observed for yellow coloured precipitate.

Test for Sterols

- **Liebermann-Burchard test (Finar, 1986):** The extract (50 mg) is dissolved in 2 ml of acetic anhydride. To this one or two drops of Conc. $H_2SO_4$is added along the side of the test tube and observed for an array of colour changes

Test for Terpenoids

- **Liebermann-Burchard test (Sofowora, 1993):** A little of extract (50 mg) was dissolved in ethanol. To it 1 ml of acetic anhydride was added followed by the addition of Conc. H_2SO_4. A change in colour from pink to violet showed the presence of terpenoids.

Test for Saponins

- **Foam Test:** The extract (50 mg) or dry powder was diluted with distilled water and made up to 20 ml. The suspension is vigorously shaken in a graduated cylinder for 15 minutes and observed for the formation of 2 cm layer thick foam.

Test for Anthraquinones

- **Borntrager's test (Sofowora, 1993):** About 0.2 g of extract to be tested was shaken with 10 ml of benzene and then filtered. 5 ml of the 10% ammonia solution was then added to the filtrate and thereafter shaken and observed for the appearance of a pink, red or violet colour in the ammoniacal (lower) phase.

Test for Proteins

- **Ninhydrin test (Yasuma and Ichikawa, 1953):** Two drops of ninhydrin solution (10 mg of ninhydrine in 200 ml of acetone) are added to 2 ml of aqueous filtrate and observed for the present of characteristic purple colour.
- **Biuret test (Gahan 1984):** An aliquot of 2 ml of filtrate is treated with one drop of 2% copper sulphate solution. To this 1 ml of 95% ethanol was added followed by excess of potassium hydroxide pellets and observed for the formation of pink ethanolic layer.

Test for Quinones

- **H_2SO_4 test (Evans, 1996):** To 1 ml of extract add 1 ml of Conc. H_2SO_4 and observed for the formation of red colour.
- **HCl test (Kokate, 2000 and Harborne, 1999):** To 1 ml of the extract 5 ml of HCl and observed for the presence of yellow colour precipitate.

3. Results and Discussion

The leaf extract of *L. inermis* have been studied for use as natural insecticides instead of organic material or other synthetic agents. Ovicidal effects of leaf extract reported in the present study confirmed their potential for control of the mosquito populations. The maximum ovicidal activity was noted in petroleum ether extract of *L. inermis* leaf in which egg hatchability was found to be totally inhibited at the concentration ranging from 150-300 ppm. At 100 ppm 11.66 and 5% egg hatchability was recorded in 48 h and 72 h (Fig 1a, b, c). Similar observations were recorded in Mullai and Jebanesan (2006) in which complete ovicidal activity was attained at 300 ppm for petroleum ether, methanol, benzene and ethyl acetate leaf extracts of two cucurbitaceous plants. Elango *et al* (2010) reported that different indigenous plant extracts showed excellent ovicidal activity against eggs of *C. quinquefasciatus*.

Petroleum ether is followed by chloroform extract in which egg hatchability was found to be totally inhibited at concentration ranging from 250-300 ppm. At 100, 150, 200 ppm zero percentage egg hatchability was recorded at 72 and 96 h. Similar observations were recorded for leaf methanol, benzene and acetone extract of *C. fistula* which exhibited excellent larvicidal, ovicidal and repellent activity against *Ae. aegypti* according to the findings of Govindarajan (2009).

Minimum ovicidal activity was exhibited by ethanol leaf extract of *L. inermis*. At higher concentration of 300 ppm the egg hatchability was found to be totally inhibited all throughout the study period. At concentration ranging from 150-250 ppm zero percentage egg hatchability was observed in 72 and 96 h (Fig 1b,c) of the study period. Similar observation were reported by Elango *et al* (2009) in which no hatchability was recorded in hexane and chloroform extract of *Andrographis paniculata* and hexane extract of *Tagetes erecta* at the higher concentration of 1000 ppm.

The data were recorded and statistical data regarding $Lc_{50,}$ Lc_{90}, UCL, LCL and chi-square values were calculated. Maximum egg mortality was exhibited by petroleum ether extract. Moderate egg mortality was recorded by chloroform extract. Probit analysis of chloroform extract exhibited the LC_{50} value for chloroform extract as 18.69. UCL LC_{50} value was recorded as 102.06 and LCL LC_{50} value was recorded as 3.42 (Table 1). Ovicidal activity of chloroform was followed by ethanol extract. Throughout the study period egg mortality was found to be 100% at higher concentration of 300 ppm. Egg mortality was found to increase at concentration ranging from 100-250 ppm. LC_{50} value for ethanol extract recorded as 20.91. UCL LC_{50} value for ethanol extract was found to be 79.23 and LCL LC_{50} value was recorded as 5.51.

Phytochemical based insecticides exhibits a variety of toxic effect in various phase of mosquito life cycle such as adulticidal, repellent, ovicidal, ovipositional, larvicidal,

pupicidal, growth and reproduction inhibition as shown in the reports of Das and Chandra, (2002), Govindarajan *et al* (2008), Govindarajan *et al* (2011b) and Chennaiappan and Kadarkarai (2008). The secondary metabolites significantly attribute to the other bioactive properties as well and determine the medicinal potential of the respective plants. The present study carried out on the *L. inermis* revealed the presence of active phytochemical compound. The leaf extracts showed the presence of various secondary metabolites viz., alkaloids, flavonoids, terpenoids, saponins, phenols, sterols, quinones, anthraquinones, etc., The result of phytochemical screening is presented in the Table 2.

The results of present study are compared with the study of Kawo and Kwa (2011) in which they reported that *L. inermis* have alkaloid, carbohydrate, saponins, sterols and tannins in different compositions. Wasim Raja *et al* (2013) reported that the phytochrmical screening of *L. inermis* showed the presences of glycosides, phytosterols, steroid, saponins, tannins and flavonoids. Similar observation were found in the present study in which ethanol extract of *L. inermis* showed the presences of phytochemical such as alkaloid, saponin, terpenoid and quinones. Secondary metabolites are known to be effective against a wide range of insect pests as well as mosquito vectors as reported by Sriwattanarungree *et al* (2008). These compounds may jointly or independently prove its efficacy against the mosquito target by its ovicidal, pupicidal, adulticidal and by inhibition of growth activity (Borah *et al.*, 2010). Ovicidal effects of leaf extracts reported in the present study thus confirmed its potential for control of the mosquito population.

4. Conclusion

Based on the present study it may be concluded that the *L. inermis* leaves have an excellent ovicidal activity against *C. quinquefasciatus*. This study could encourage the search for an alternative to synthetic insecticides. Plant extracts have potential to be developed as new safe control product against the vector mosquito. According to the early reports the phytochemical constituents are responsible for the therapeutic properties of plants. Indiscriminate use of synthetic chemicals and insecticides to control the mosquitoes in the natural habitats has developed strong resistances and over use of these insecticides has led to inevitable environment degrading effects.

Research has now shifted to the use of botanical insecticides which are environmentally safe and non-hazardous to non-target organism. The result of this study also demonstrates the potential of new alternative source of mosquito ovicides which are generally free from adverse effects and are very promising to be developed into a new, effective and inexpensive approach to control *C. quinquefaciatus*. It may be conclude that the extracts of *L. inermis* is most potent in the treatment against *C. quinquefasciatus* mosquito. Based on these results the petroleum ether extract of *L. inermis* could be used in vector mosquito control. The results thus encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other plants.

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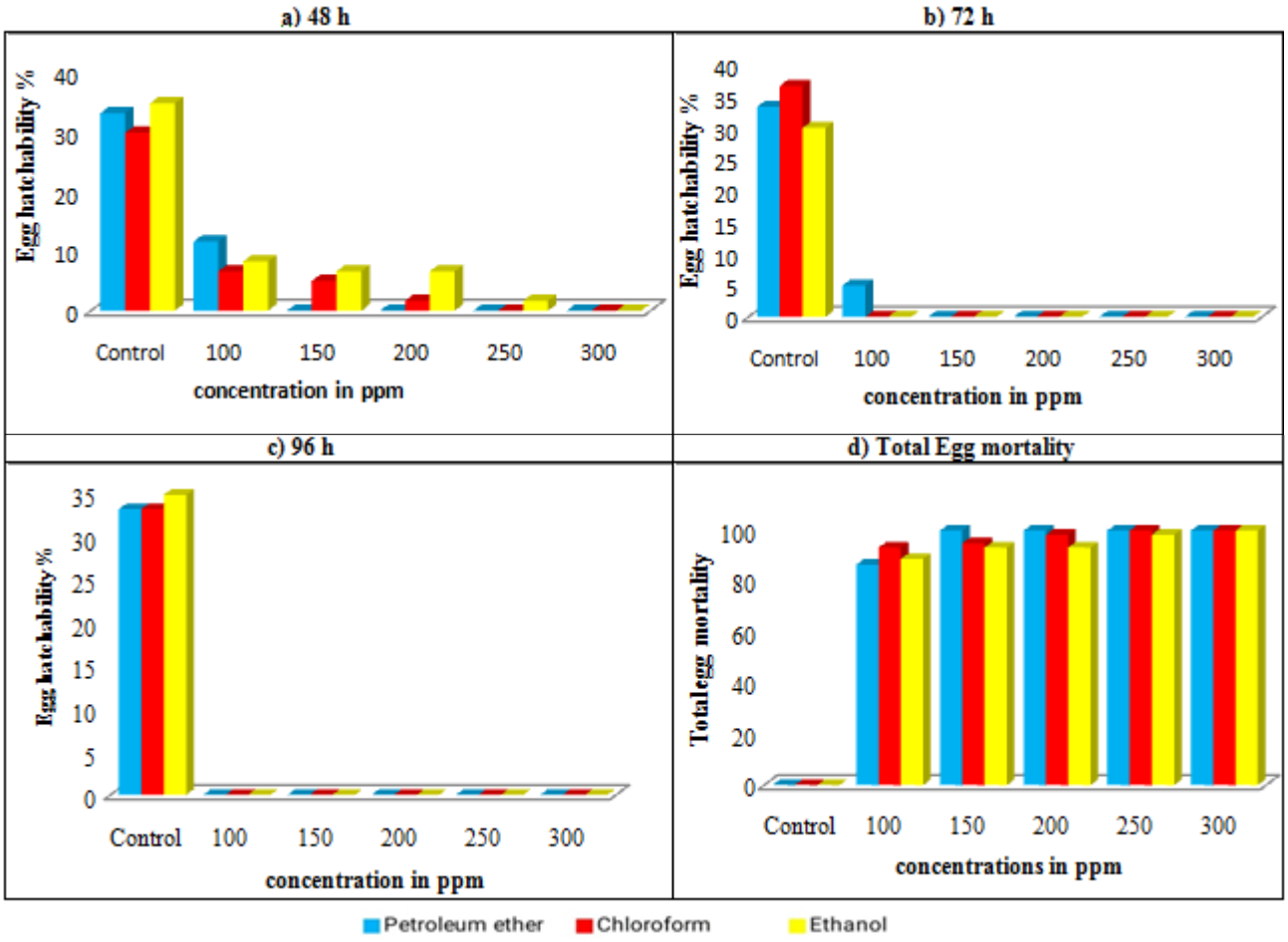

Figure 1: Effect of *Lawsonia inermis* leaf extracts on egg hatchability of *C.quinquefasciatus*

Table 1: Lethal concentration of leaf extracts of *Lawsonia inermis* against eggs of *C. quinquefasciatus*

Solvents Used	Log LC_{50}	Log LC_{70}	Log LC_{90}	LC50 (ppm)	LC_{70} (ppm)	LC_{90} (ppm)	Regression Equation	95% Confidence Limits				χ^2	SE
								UCL (ppm)		**LCL (ppm)**			
								LC_{50}	**LC_{90}**	**LC_{50}**	**LC_{90}**		
Petroleum ether	1.65	1.92	2.29	45.68	83.34	198.49	Y=1.66+2.00X	218.7	341.9	9.54	115.2	8.1	1.47
Chloroform	1.51	1.75	2.09	32.68	56.37	123.79	Y=1.64+2.21X	1.48	308.4	7.19	1.64	8.33	12.4
Ethanol	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2: Phytochemicals present in the leaf extracts of *Lawsonia inermis*

Sl. No.	Constituents	*L. inermis leaf*		
		Petroleum ether extract	**Chloroform extract**	**Ethanol extract**
1	Alkaloids	-	-	+
2	Tannins	+	-	-
3	Phenols	-	-	-
4	Flavonoids	-	-	-
5	Sterols	+	+	-
6	Terpenoids	+	+	+
7	Saponins	+	+	+
8	Anthroquinones	+	-	-
9	Proteins	+	+	-
10	Quinones	+	-	+