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Research Article

PHYTOCHEMICAL PROFILE AND LARVICIDAL ACTIVITY OF *PHYLLANTHUS NIRURI* LEAVES EXTRACT AGAINST MOSQUITO LARVAE

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ABSTRACT

Phytochemical analysis and mosquito larvicidal effect of leaf extracts of *Phyllanthus niruri* was studied against malaria, filaria and dengue vectors of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. The phytochemical analysis was assessed by phytochemical screening tests. The larvicidal activity of *Phyllanthus niruri* leaves extract was tested against mosquito larvae following the WHO protocol. The mortality was observed at 24h after treatment and the data were subjected to probit analysis to determine the lethal concentration (LC_{50} and LC_{90}) to kill 50 and 90 percent of the treated larvae of the tested species. The study has revealed the presence of bioactive chemicals such as alkaloids, flavonoids, terpenoids, phenolic compounds, saponins, tannins, cardiac glycosides and also coumorins in the leaves extract. The results showed that the *Phyllanthus niruri* leaves extract was highly effective against the larvae of *Anopheles stephensi* (LC_{50} = 4.46 ppm and LC_{90} = 9.25 ppm) and against *Aedes aegypti* (LC_{50} = 11.92 ppm and LC_{90} = 14.60 ppm). The study results suggested that *Phyllanthus niruri* leaves can be applied as an ideal potential larvicide against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. This is an ideal ecofriendly approach for the control of the malaria, filarial and dengue vectors.

Keywords: *Phyllanthus niruri*, Malaria, Filarial, Dengue, Mosquito vectors.

INTRODUCTION

Mosquitoes are the chief and major blood sucking vectors transmits pathogens and parasites which causes devastating impact on human beings. Mosquito borne diseases have an economic impact, including commercial loss and outputs of labor, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector borne diseases (Fradin & Day, 2002). *Anopheles stephensi* Liston is the primary vector of malaria in India and other Asian countries. Malaria remains one of the most prevalent diseases in the tropical world. With 200 million to 450 million infections annually worldwide, it causes up to 2.7 million deaths (WHO, 2012). *Culex quinquefasciatus* (Say.) acts as a vector for filariasis in India. Human filariasis is a major public health hazard and remains a challenging socioeconomic problem in many of the tropical countries (Mullai & Jebanesan, 2007; Udonsi, 1986). *Aedes aegypti* (L.) is generally known as a vector for dengue is widely distributed in tropical and subtropical zones. Dengue fever has become an important public health problem as the number of reported cases continues to increase, especially with more severe forms of the disease such as dengue hemorrhagic fever and dengue shock syndrome (Pancharoen *et al.*, 2002). Globally, these diseases remain endemic and its control is a most important goal for improved worldwide health.

Due to faster onset of action, synthetic larvicides are used as the first line of choice. However, the continuous discriminate use of synthetic larvicides may lead to the development of resistance and permanent residual effect on the ecosystem, which can be detrimental to animals, including humans (Yadav & Sahu, 2017). Bacterial biopesticides such as *Bacillus sphaericus* was also reported high level resistance to mosquito vectors (Poopathi *et al.*, 2014). Under these circumstances, the Environmental Protection Act in 1969 has framed a number of rules and

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regulations to check the application of chemical control agents in nature (Bhatt & Khanal, 2009). It has prompted researchers to look for easily biodegradable alternative larvicides to control vector mosquitoes. The pharmacological and larvicidal properties of plants have been recognized in many parts of the world, especially in India. Where plant materials are easily available and their use in health practices is a tradition. Available sources of indigenous plant material can possibly be used to control mosquitoes in and around human habitation after screening them in the laboratory. Most of plants defensive components are biodegradable without causing residual effects on the ecosystem.

Phyllanthus niruri is a plant of the family *Euphorbiaceae* which are found in tropical and subtropical countries. In India, *Phyllanthus niruri* is widely distributed as a weed in cultivated and waste lands (Joseph & Raj, 2011). It is used in several health problems such as diarrhoea, dysentery, dropsy, jaundice, intermittent fevers, urogenital disorders, scabies and wounds (Khatoon & Gopalakrishna, 2004; Sen & Batra, 2013; Ushie *et al.*, 2013a). Therefore, an attempt has been made in the present study to identify the extract of *Phyllanthus niruri* with potential to control vector mosquitoes

MATERIALS AND METHODS

Collection of Plant leaves

The healthy leaves of Kizhanelli (*Phyllanthus niruri*) were arbitrarily collected from the Mathagadipet region of Puducherry, India during the rainy seasons between September and November 2016. The collected plant was identified with taxonomically (Ushie *et al.*, 2013b) and confirmed at Department of Botany, KMCPGS, Puducherry. The leaves were washed with double distilled water and dried in shade for 3 to 5 days with the room temperature (28±2) in the Department of Zoology. This dried plant leaves were powdered by mechanical and stored in air tight container to protect from humidity and light for solvent extraction.

Preparation of Solvent extraction

A portion of the dried leaves powder was soaked in the conical flask containing ethanol (Analytical grade) and wrapped with aluminum foil for 72 hours with occasional shaking. After 72 hours, the extracts were filtered using Whatman filter paper No: 1. the solvent was removed from the extract by vacuum distillation. The concentrated *Phyllanthus niruri* leaves extract was dried and stored at 4^{o}C until use. This extract was subjected for phytochemical analysis and mosquito larvicidal assay study.

Phytochemical analysis

Preliminary phytochemical screening of plant methanol extract was carried out to detect the phyto-constituents using standard conventional protocols (Harborne, 1998).

Test for alkaloids (Wagner's reagent)

A fraction of extract was treated with 3-5drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of water) and observed for the formation of reddish brown precipitate (or colouration).

Test for flavonoids

Extract was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless indicates the presence of flavonoids.

Test for terpenoids

Salkowski's test: extract was treated with 2 ml of chloroform and filtered. The filtrate was treated with few drops of conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicated the presence of triterpenoids.

Test for phenolic compounds

Ferric chloride test: To 1 ml of extract, few drops of 0.5% ferric chloride solution was added. Formation of bluish black colour indicated the presence of phenolic compounds.

Test for saponins

1 ml aliquot of floral extract was combined with 5 ml distilled water at 60°C, then, shaken for 2 min, as saponins are known to possess frothing activity, the volume of froth produced in this experiments was observed and recorded every 10 min for a period of 30 min

Test for tannins (Braymer's test)

2 ml of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

Tests for glycosides

To 2 ml of extract, add 3 ml of $CHCl_3$ and 10% ammonia solution formation of pink colour indicating presence of glycosides.

Test for cardiac glycosides (Keller Kelliani's test)

About 5 mL of each extract was treated with 2 mL of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully under layed with 1ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of de-oxy sugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

Test for coumarin

To 2 mL of test solution few drops of alcoholic sodium hydroxide were mixed well and the formation of yellow colour indicates the presence of Coumarin.

Mosquito larvicidal assay

Larvicidal assays were carried out at room temperature (25±3°C) as per the standard methods (WHO, 1982, 1985; Poopathi *et al*. 2015). For bioassays, a homogeneous stock solution from dried leaves extract prepared (100 mg/l), and mosquito toxicity assay was carried out in disposable wax-coated paper cups (350 ml capacity). Serial dilutions were prepared by adding the appropriate volume of stock solution (0.07 - 25 mg/l) in 300 ml of distilled water, into which 25 early third instar larvae from the respective mosquito species were introduced separately. Food supplement (dog biscuit and yeast, 2:1) was provided to the mosquito larvae, and the mortality was monitored after 24 h. Moribund larvae (if any) in the replicates were counted as dead. Control mortality (if any) was corrected (Abbott, 1925) and the percentage mortality was calculated as follows:

$$\text{Percentage mortality} = \frac{\text{Number of larave died}}{\text{Total number of larave exposed}} \times 100$$

Statistical analysis

Statistical analysis of the study records were carried out withthe help of the SPSS package to find the mean, standard deviation (±SD), LC_{50}, LC_{90}, regression equation and Chi-square (χ^2) values. Results with $p<0.05$ were considered to be statistically significant.

RESULT AND DISCUSSION

Thephytochemical composition of *Phyllanthus niruri* leaves ethanolic extract show in Table 1. It revealed the presence of bioactive compounds such as alkaloids, flavonoids, terpenoids, phenolic compounds, saponins, tannins, cardiac glycosides and also coumorins. The presence of carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins in the plants extract having mosquito larvicidal activity (Kumar & Maneemegalai, 2008). Alkaloid derived from the tropical vine *Triphyophyllum peltatum* (Dioncophyllaceae), was found to have larvicidal activity against the malaria vector *Anopheles stephensi* (Francois *et al.*, 1996). Similarly, a piperidine alkaloid from *Piper longum* fruit was found to be active against mosquito larvae of *C. pipiens* (Lee *et al.*, 2000).

Table 1. Phytochemical screening of *Phyllanthus niruri* leaves ethanolic extract.

Phytoconstituents	Ethanolic extract of *Phyllanthus niruri* leaves
Alkaloids	Presence
Flavonoids	Presence
Terpenoids	Presence
Phenolic compounds	Presence
Saponins	Presence
Tannins	Presence
Glycosides	Presence
Cardiac glycosides	Presence
Coumorins	Presence

(Pelah *et al.*, 2002) reported the use of commercial saponin from *Quillaja saponaria* bark as a natural larvicidal against *Aedes aegypti* and *Culex pipens*. (Wiesman & Chapagain, 2006) reported that saponin extracted from the fruit of *Balanites aegyptiaca* showed 100% larvicidal activity against *A. aegypti* mosquito larvae. Morrissey & Osbourn, (1999) have suggested that the saponin molecules interact with the cuticle membrane of the larvae, ultimately disarranging the membrane could be the most probable reason for the larval death. The deficiency of dissolved oxygen and active presence of the antioxidant saponin molecule might be the reason for larval death. Prenylated xanthones, tetracyclic phenols and saponins are reported to be effective in controlling mosquito *A. aegypti*, the vector of yellow fever (Marston & Redwood, 1993). Isoflavonoids from tubers of *Neorautanenia mitis* had larvicidal effect against the malaria and filariasis transmitting mosquitoes, *Anopheles gambiae* and *C. quinquefaciatus*, respectively (Joseph, 2004). Fortunately in all compounds such as phenolic compound, flavonoids, terpenoids, saponin, cardiac glycosides and steroids were found in *P. niruri*. There might be the core reason to have the dominant larvicidal activity.

The larvicidal activity of the *Phyllanthus niruri* leaves ethanolic extract against mosquito larvae are noted and presented in Table 2 and Fig. 1.The leaves extract was highly effective against the larvae of *Anopheles stephensi* (LC_{50} = 4.46 ppm and LC_{90} = 9.25 ppm), against *Aedes aegypti* (LC_{50} = 11.92 ppm and LC_{90} = 14.60 ppm) and moderate effective against *Culex quinquefaciatus* larvae (LC_{50} = 47.86 ppm and LC_{90} = 93.48 ppm).

Table 2. LC_{50} /LC_{90} value of ethanolic extract of *Phyllanthus niruri* leaves against mosquito larvae.

Mosquito species	Intercept	Slope	LC_{50} (PPM) (90% UCL – LCL)	LC_{90} (PPM) (90% UCL – LCL)	χ^2 (*df*= 6)
A. stephensi	1.75	2.38 ± 0.06	4.46 (3.93 - 5.05)	9.25 (7.09 – 12.06)	11.1
C. quinquefasciatus	1.91	2.39 ± 0.06	47.86 (42.53 - 53.85)	93.48 (72.54 – 120.48)	16.1
A. aegypti	6.35	10.75 ± 0.02	11.92 (11.49 - 12.37)	14.60 (13.62 – 15.6)	1.3

The control has nil mortality. Values were significant at the P<0.05 level.

LC_{50}: lethal concentration that kills 50% of the exposed larvae, LC_{90}: lethal concentration that kills 90% of the exposed larvae, UCL: upper confidence limit (95% fiducial limit), LCL: lower confidence limit (95% fiducial limit), χ^2: chi-square, *df* : degrees of freedom.

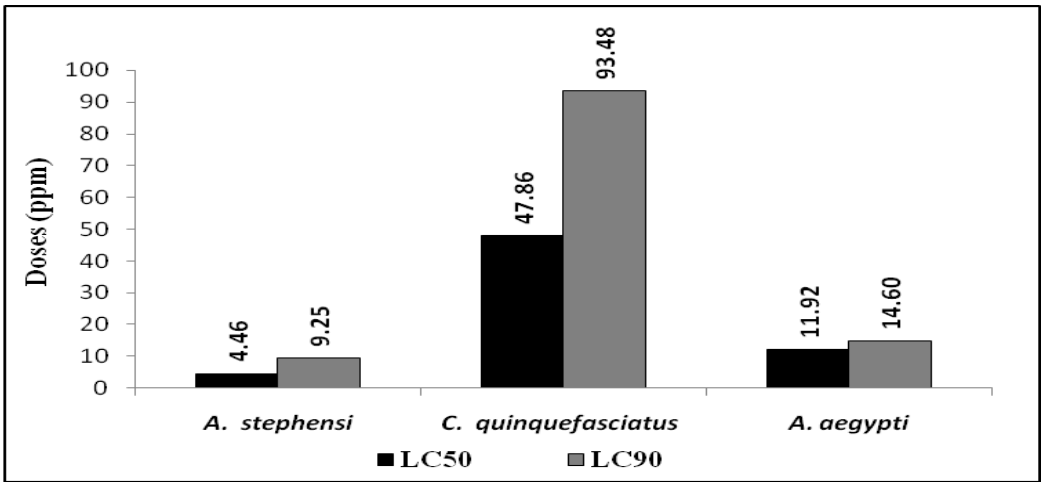

Figure 1. Histogram showing the LC_{50} and LC_{90} values of *Phyllanthus niruri* leaves against mosquito larvae.

The methanolic fraction of leaves of *Mentha piperita*, *Phyllanthus niruri*, *Leucas aspera*, and *Vitex negundo* against larvae of *Culex quinquefasciatus* (Pandian *et al*., 1994). (Jang *et al.*, 2002) have reported that the methanol extracts of *Cassia obtusifolia*, *Cassia tora*, and *Vicia tetrasperma* exhibited more than 90% larval mortality at 200 ppm on *A. aegypti* and *C. pipiens*. The leaf extract of *Solanum trilobatum* reduced egg laying by gravid females of *Anopheles stephensi* from 18% to 99% compared with ethanol-treated controls at 0.01, 0.025, 0.05, 0.075 and 0.1% (Rajkumar & Jebanesan, 2005). Mullai & Jebanesan, (2007) have reported that ethyl acetate, petroleum ether, and methanol leaf extracts of *Citrullus colocynthis* and *Cucurbita maxima* showed LC_{50} values of 47.58, 66.92, and 118.74 ppm and 75.91, 117.73, and 171.64 ppm respectively, against *C. quinquefasciatus* larvae. Karunamoorthi *et al*. (2008) were evaluated the petroleum ether extracts of the leaves of *Vitex negundo* for larvicidal activity against larval stages of *C. tritaeniorhynchus* in the laboratory with LC_{50} and LC_{90} values of 2.4883 and 5.1883 mg/l, respectively. The ethanolic leaf extract of *Cassia obtusifolia* had significant larvicidal effect against *An. stephensi* with LC_{50} and LC_{90} values of 52.2 and 108.7 mg/l, respectively (Rajkumar & Jebanesan, 2005).

Larvicidal activity of crude extract of *Sida acuta* against three important mosquitoes with LC_{50} values ranging from 38 to 48 mg/L. The crude extract had strong repellent action against three species of mosquitoes as it provided 100% protection against *An. Stephensi* for 180 minutes followed by *Ae. aegypti* (150 min) and *Cx. quinquefasciatus* (120 min) respectively (Govindarajan, 2010). The present study revealed that *Phyllanthus niruri* leaves extract contains phytochemicals leading to significant mortality against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* developmental stages of mosquito life cycle. The study further revealed that the methanolic extract of leaves of *Phyllanthus niruri*is highly

effective against the mosquito larvae when compared to other plant extracts like *Trigonella Foenum graecum, Senna auriculata,* etc.

CONCLUSION

The present study revealed that *Phyllanthus niruri* leavespossesses phytochemicals such as alkaloids, flavonoids, terpenoids, phenolic compounds, saponins, tannins, cardiac glycosides and coumorins. *Phyllanthus niruri* leaves have significant larvicidal activity against mosquito larvae. The study results also suggested that *Phyllanthus niruri* leaves can be applied as an ideal potential larvicide against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. This is an ideal ecofriendly approach for the control of the malaria, filarial and dengue vectors. The extracts from this plant leaves may be useful for development of new bio-rational mosquito larvicidal pesticides. However, further investigations are advocated to identify the effective components and their mechanisms of actions of this species.

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