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**Research Article**

# PHYTOCHEMICAL SCREENING AND LARVICIDAL EFFICACY OF *TRIGONELLA FOENUM-GRACEUM* LEAVES EXTRACT ON THE MOSQUITOES LARVAE

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**ABSTRACT**

Mosquitoes are primary vector for malaria, dengue haemorrhagic fever, filariasis, yellow fever, chikungunya and other severe infectious diseases. The aim of this study is to evaluate phytochemical analysis and larvicidal activity of *Trigonella foenum-graceum* leaves against mosquito larvae. The larvicidal activities of *T. foenum-graceum* leaves extract on the different mosquito larvae were carried out using WHO protocol. In the present studies phytochemical screening of the *T. foenum-graceum leaves* extract shows the presence of alkaloids, flavonoids, saponins, tannin, glycosides and steroid. The infrared spectral data obtained revealed the presence of characteristic functional groups of alcohol, hydroxyl, alkanes, alkenes, alkynes and aliphatic amines etc. The results showed that *T. foenum-graceum* leaves extract has significant larvicidal activity against *Aedes aegypti* and *Anopheles stephensi.*

**Keywords:** Larvicidal activity, Mosquito, *Trigonella foenum-graceum, Aedes aegypti.*

## INTRODUCTION

Mosquitoes constitute a major public health problem by transmit serious human diseases like malaria, dengue haemorrhagic fever, filariasis, chikungunya and yellow fever causing millions of deaths globally. Mosquitoes in the larval stages are ideal attractive targets for insecticides because they are breeds in water which will be easy to deal with them in this habitat. Thus, larvicides play a vital role in controlling mosquitoes in their breeding sites. Even though various control measures are in approach, the synthetic chemicals insecticidal such as organophosphates, organochlorines, pyrethroids and carbamates have proven to be the most important effective method to control mosquitoes worldwide. Their extensive and indiscriminate uses fostered not only environmental and health concerns but also development of resistance by mosquitoes and harmful effects to beneficial insects. This situation has forced scientists to find alternate and ecofriendly compounds with potent anti-mosquito activity. Plant based larvicides are less toxic, delay the development of resistance because of its new structure and easily biodegradable (Ignacimuthu, 2000). Therefore, there has been an increased attention, in recent years, to the use of natural larvicide.

*Trigonella foenum-graceum* (Fenugreek) is a self-pollinating crop, which is a native plant of the Indian subcontinent and the Eastern Mediterranean region. The crop extends to central Asia and North Africa, and more recently it has been successfully grown in Central Europe, UK, and North America. India is the largest producer of fenugreek in the world where Rajasthan, Gujarat and Uttanchal are producing states (Abdul *et al*., 2008). It belongs to the Papilionaceae section of the family Leguminosae. Fenugreek has been used traditionally to treat diabetes (Yu *et al*., 2002), coughs, congestion, bronchitis, fever, high blood pressure, headaches, migraines, diarrhea, anemia (Ng *et al*., 2007), flatulence, irregular menstrual cycles, analgesic, inflammation and arthritis (Vyas *et al*., 2008) and as an appetite stimulant. The aim of this study was to evaluate the mosquito larvicidal activity of *T. foenum-graceum* leaves extract by WHO protocol with preliminary phytochemical screening of the extract.

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## MATERIALS AND METHODS

### Sample collection

The leaves of *T. foenum-graceum* were collected from the Embalam region of Puducherry, India and taxonomic identification was made by plant biologist. The leaves were cleaned and air dried at room temperature in the Department of Zoology until constant weight was attained. The dried leaves were pulverized into fine powder using electric blender.

### Preparation of Solvent extraction

A portion of the dried leaves powder was soaked in the conical flask containing methanol (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 *T. foenum-graceum* 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 screening

The phytochemical screening was the qualitative analyzed in accordance with Peach and Tracey (1955) to determine the secondary metabolites present in the extract.

### (a) Test for Alkaloids

**Wagner's test:** 1.0 g of iodine and 2.0 g of potassium iodide were dissolved in 5 ml sulphuric acid and solution was diluted to 100 ml. 10 ml extract was acidified by adding 1.5% v/v HC1 and a few drops of Wagner's reagent. Formation of yellow or brown precipitate confirmed the presence of alkaloids.

### (b) Test for flavonoids

In a test tube containing 0.5 ml of the extract, 5-10 drops of dilute hydrochloric acid and small piece of Zn or Mg was added and the solution was boiled for few minutes. In the presence of flavonoids, reddish pink or dirty brown colour was produced.

### (c) Test for saponins

In a tube containing about 5.0 ml of extract, a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3 minutes. A honey comb like froth was formed and it showed the presence of saponins.

### (d) Test for Tannins

To 1.2 ml of the extract, few drops of 1% solution of lead acetate were added. A yellow or red precipitate was formed, indicating the presence of tannins.

### (e) Test for Glycosides

A small amount of extract was dissolved in 1.0 ml of water and then aqueous sodium hydroxide solution was added. Formation of a yellow colour indicates the presence of glycosides.

### (f) Test for Steroids

To 1.0 ml of extract, 1 ml of concentrated sulphuric acid was added followed by the addition of 2.0 ml of acetic anhydride solution. A greenish colour developed and it turned blue. It indicates the presence of steroids.

### FTIR Spectroscopic Analysis

Fourier transform infrared Spectrophotometer (Shimadzu, IR prestige-21) was used to identify the characteristic functional groups in the leaves extract. A small quantity (5 mg) of the leaves extract was dispersed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The sample was scanned for transmittance within 4000 $cm^{-1}$ to 400 $cm^{-1}$ (mid IR region). The IR spectrum was printed with the individual peaks labeled with their corresponding wavelengths.

### Mosquito larval collection

Larval samples will be collected from breeding places by using 350 ml standard larval dipper. Dips will be taken gently with a 2 to 3 minutes pause, to allow the mosquito larvae to move freely in the air water interface and henceforth a minimum of 5 to 10 dips will be made. Field collected larvae will be transported to the laboratory for identification of *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti* species.

### Mosquito larvicidal assay

Larvicidal assays were carried out at room temperature (25±3°C) as per WHO protocol (WHO 1982, 1985). 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). Dilutions were prepared by adding the appropriate volume of stock solution (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 and 48 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:

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

**Statistical analyses**

The larvicidal activity of *T. foenum graceum* leaves extract were carried out in six replicates and results are presented as means ± standard deviation (SD).

## RESULT AND DISCUSSION

The phytochemical constituents of *T. foenum graceum* leaves extract as shown in the Table 1. The biochemical test for alkaloids, flavonoids, saponins, tannin, glycosides and steroid were positive in the leaves extract. Imaga *et al.* (2010) indicated the presence of alkaloid, flavonoid, saponins and glycosides in the extract of *Carica papaya* leaves and these compounds have been found to possess high larvicidal activities against different species of mosquitoes (Quevedo *et al.*, 2012).

The FTIR spectrum of *T. foenum graceum* leaves extract exhibited absorption in the range from 3387.11 $cm^{-1}$ to 516.28 $cm^{-1}$ (Figure 1). The spectrum exhibited a broad band around 3387.11 $cm^{-1}$ assigned to Alcohol and hydroxyl group (O-H) stretching. This may also indicated the presence of phenol and flavonoid. The sharp peak observed at 2928.89 $cm^{-1}$ shows the presence of alkanes group (C-H). Another sharp peak at 1643.74 $cm^{-1}$ attributed to alkenes (C=C). The peak at 1380.51 $cm^{-1}$ indicates the presence of alkanes (C-H). Sharp peak observed at 1047.27 $cm^{-1}$shows the presence of aliphatic amines (C-N). The peaks observed at 838.68, 769.14, 668.65, 525.00 and 516.28 $cm^{-1}$ represents C-H stretch of alkynes. This FTIR spectrum analysis result of *T. foenum-graceum* leaves shows the presence of characteristic functional groups of alcohol, hydroxyl, alkanes, alkenes, alkynes and aliphatic amines.

The results of larvicidal activity of *T. foenum-graceum* leaves extract against *C. quinquefasciatus, A. stephensi* and *A. aegypti* for the duration of 24 and 48 hours were shown in Table 2. The highest mortality (100%) was detected against *A. aegypti* at the both 24 and 48 h. Significant activity was observed against *A. stephensi* (93-97% after 24 and 48 h exposure). *T. foenum graceum* leaves extract exhibits considerable (59-80%) larvicidal activity against *C. quinquefasciatus.*

**Table 1.** Phytochemical constituents of *Trigonella foenum-graceum* leaves extract.

| Sl. No. | Phytoconstituents | Methanolic extact of *T. foenum-graceum* leaves |
|---|---|---|
| 1 | Alkaloid | + |
| 2 | Flavonoid | + |
| 3 | Saponin | + |
| 4 | Tannins | + |
| 5 | Glycosides | + |
| 6 | Steroid | + |

+ = present of constituents.

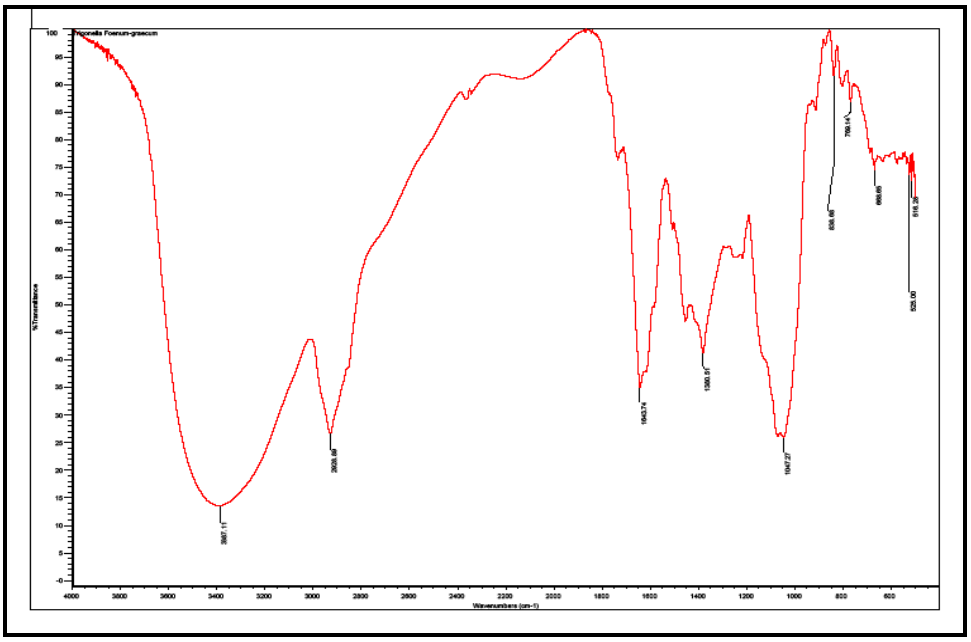

**Figure 1.** FTIR Spectroscopic analysis of *T. foenum-graceum* leaves extract.

**Table 2.** Mortality percentage of *T. foenum-graceum* leaves extract against mosquito larvae.

| Sl. No. | Mosquito species | Duration | Number of larvae exposed | Mean value of Mortality | Percentage of Mortality |
|---|---|---|---|---|---|
| 1 | *Culex quinquefasciatus* | 24 hours | 25 | 14.8 ± 4.2 | 59% |
| 2 | *Anopheles stephensi* | | 25 | 23.2 ± 2.3 | 93% |
| 3 | *Aedes aegypti* | | 25 | 25.0 ± 0.0 | 100% |
| 1 | *Culex quinquefasciatus* | 48 hours | 25 | 20.0 ± 0.6 | 80% |
| 2 | *Anopheles stephensi* | | 25 | 24.2 ± 1.6 | 97% |
| 3 | *Aedes aegypti* | | 25 | 25.0 ± 0.0 | 100% |

Control – Nil mortality, Mean value of six replicates ± SD.

Using synthetic insecticides are effective but they create many problems like development of insecticide resistance (Lin *et al*., 2005). Therefore, application of indigenous plant based products could provide standardized measure for protection to the human population against various disease caused by mosquitoes. Many approaches that have been developed to control the mosquito menace. One such approach to prevent mosquito borne disease is to kill at its larval stage. Many studies made use of plant extracts for mosquito control approach. Kamaraj *et al*. (2011) reported that plants derived extracts using different solvents crude extracts have potential larvicidal activity. To evaluate the potential larvicidal activity of the plant preliminary screening is a good measure (Ali *et al.,* 2012). Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future (Kamaraj *et al*., 2009). Howard *et al.* (2007) revealed that larval control can be the effective appropriate way in controlling the mosquitoes in breeding habitats, which are man-made.

Yenesew *et al*. (2003) reported that *Millettia dura* chloroform extract shows higher larvicidal activity against the second instar larvae of *A. aegypti.* Ansari *et al.* (2005) was observed the larvicidal activity of *Pinus longifolia* oil against *A. stephensi*, *A. aegypti* and *C. quinquefasciatus.* Theextracts of *Jatropha curcas* and *Euphorbia tirucalli* were highly effective against the larvae of *A. aegypti* (Rahuman *et al*., 2008). Mullai and Jebanesan (2007) stated that four different extracts of *Cucumis pubescens* leaf was effective on larvicidal effect against *A. stephensi, C. quinquefasciatus* and *A. aegypti.* The ethanol extract of *Annona squamosa* leaf was effective in larvicidal activity against *C. quinquefasciatus* (Fahd *et al*., 2013). The present study revealed that *T. foenum graceum* leaves extract contains phytochemicals leading to remarkable mortality against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* developmental stages of mosquito life cycle.

## CONCLUSION

The present study revealed that *T. foenum graceum* leaves possess phytochemicals such as alkaloids, flavonoids, saponins, tannin, glycosides and steroid. The infrared characterization revealed the presence of aliphatic as well as aromatic compounds. *T. foenum-graceum* leaves have significant larvicidal activity against *A. aegypti* and *A. stephensi.* On the basis of the present investigation results *T. foenum graceum* leaves extract contains potent larvicidal bioactive principles. Further investigation of the bioactive compounds in this plant leaves extract that might possess good larvicidal properties when it will be isolated in pure form.

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