



**REPELLENT ACTIVITY AND PHYTOCHEMICAL SCREENING OF FRUIT  
EXTRACTS TAMARINDUS INDICA L. AGAINST CULEX QUINQUE FASIATUS**

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**ABSTRACT**

Mosquitoes are the major cause for many diseases because of blood feeding mode of life style to living being worldwide. Which bites painful, irritate, swelling, major nuisance and causing sleepless nights. The adult females of *Culex quinquefasciatus* is a persistent biters and it emerges at evening dark prefers to attack at shadows, because they need blood for nutrients. The fruit and leaf extract of *T. indica* L. have higher repellent efficacy against the vector *C. quinquefasciatus*. These extracts will provide further clarity about the nature of these bioactive compounds which could become an alternative to the conventional insecticides used for repelling annoying mosquito species. The results showed that the ethanolic extract of fruit pulp of *T. indica* L. is found to be effective against *C. quinquefasciatus* and 100% protection time till 90 minutes at the concentration of 1.0, 2.5 and 5.0 mg/cm<sup>2</sup> followed by petroleum ether extract showed maximum repellency at 5.0 concentrations and it showed 99.33% in the first 30 minutes.

**KEYWORDS:** *Culex Quinquefasciatus*, Repellent Efficacy, *Tamarindus Indica*.

**INTRODUCTION**

Mosquitoes are among the best known groups of insect, because of their importance to human as pests and vectors of some of the most distressing human diseases. The diseases transmitted through mosquito are the major cause of human death worldwide. No part of the world is free from vector borne diseases.<sup>[7]</sup> Mosquito-borne diseases, such as malaria, filariasis, dengue, haemorrhagic fever and chikunguniya are still imposing to be major public health problem in the Southeast Asian countries because of their tropical or subtropical climate.<sup>[9]</sup>

WHO has declared mosquito as “Public enemy number one”. Mosquito borne diseases are prevalent in more than 100 countries across the world, infects 700 million people every year globally and 40 millions of the Indian population. In 2008, there were 243 million cases, and an estimated 8, 63,000 deaths attributed to malaria.<sup>[18]</sup> Plants have been known to relieve various diseases in traditional medicine and Ayurveda. Secondary metabolites are responsible for medicinal activity of plants.<sup>[2]</sup> Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. Plant products have been part of phytomedicines since time immemorial. This can be

derived from barks, leaves, flowers, roots, fruits and seeds.<sup>[10]</sup>

Mosquitoes are known for their peculiar characters of biting and blood feeding mode of nutrition and its irritating behaviour. Mosquito borne diseases are challenging to mankind. Mosquitoes gained resistant to different kinds of chemical insecticides because of continuous usage and pollution. Plants are boons for many diseases. Plant based mosquito repellents and larvicides are safer than synthetic products. These plants based ingredients are eco friendly to non target organisms.<sup>[3]</sup> *Tamarindus Indica* L. has antibacterial properties against *burkholderia pseudomallei*, *Klebsiella pneumonia*, *Salmonella paratyphi*, *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli* and *staphylococcus aureus*.<sup>[5]</sup>

**MATERIALS AND METHODS**

**Collection of samples:** The fruit chosen for the present study (*Tamarindus indica* Linn) is collected from Ukkadam area, Coimbatore district. The fruits were washed with tap water to remove the direct. The fruit pulp was dried and homogenized to a fine powder and stored in sterile air-tight container until further use. The selected fruit sample was identified as *Tamarindus indica* L. (BSI/SRC/5/23/2017/Tech/3267). It was authenticated by Tamilnadu Agricultural University, Coimbatore.

### Preparation of the extracts

10 g of each of the fruit was weighed using an electronic balance (Denver XS-210) and made into packets using zero haze filter paper (A Grade, SD's). These powders were subjected to extraction with 500 ml of the solvents for 8 h using a Soxhlet apparatus. Petroleum ether (60-80°C) extraction was followed by chloroform extraction and ethanol extraction so that the powder subjected to extraction with solvents of increasing polarity.

### Laboratory culture of larvae

Hay infusion method was adopted for culturing mosquito larvae. Hay was taken, cut into small pieces and boiled in 5 litres of water for 20 minutes. After cooling, this water was poured into buckets and kept in different areas where mosquitoes were abundant. After one or two days eggs were laid by female mosquitoes in clusters forming an egg raft. The egg rafts were collected and maintained in the laboratory. The third instar larvae were collected, reared in enamel trays containing culture medium and provided with powdered dog biscuits and yeast in the ratio of 3:1 as the nutrient source. Immediately after molting, the fourth instar larvae were introduced into beakers containing 200 ml of water and used for the bioassay studies.

### Laboratory culture of adult mosquitoes

Adult mosquitoes were reared in wooden cage (30cm ×30cm×30cm) and daily provided with sponge pieces sealed with 10% of sucrose solution for a period of 3- 4 days. After emergence mosquitoes were held at 28±2 °C, 70% - 85% relative humidity with a photo period of 14:10 light and dark photo period cycle. Three day blood starved *C. quinquefasciatus* mosquitoes were used for repellent bioassays studies.

### Bioassay studies

A present study was carried out to access the repellent efficacy of fruit extracts of selected plant against three day old blood starved *C. quinquefasciatus* mosquitoes. Effective doses were determined first and then detailed. Investigation carried out concentration of 1.0, 2.5 and 5.0mg/cm<sup>2</sup> were used for determining the repellent efficacy of the fruit pulp *T. indica* L.

### Experimental design

The experimental setup consists of three treatments each with replications for fruit extracts. Simultaneously control was also maintained. The repellent study was followed by the method of WHO. Three days old blood starved mosquito female *C. quinquefasciatus* were kept in the net cage (45cm ×30cm×45cm). The arms had no contact with lotions, perfumes, gels, powders and soaps on the day of assays. Dorsal side of the right arms was treated with extracts, a left arm was kept as control and the remaining area was covered by rubber gloves. Crude extracts was applied at 1.0, 2.5 and 5.0mg/cm<sup>2</sup> separately in the exposed area of the forearm. The control and treated arms were introduced continuously into the mosquito net cage and gently tapping the sides of the

cage. The mosquitoes were activated. The test was conducted at each extracts by inserting the treated and control arm into the same cage for one full minute of every five minutes. The mosquitoes that landed on the hand were recorded and then shaken off before sucking any blood and making out five minutes protection from mosquito bite.

### Test for repellent activity

The percentage of repellency was calculated by the following formulae,

$$\text{Percentage of repellency} = \frac{(Ta - Tb)}{Ta} \times 100$$

here,

Ta is the number of mosquitoes in the control arm.

Tb is the number of mosquitoes in the treated arm.

### Statistical analysis

The data on bioassay studies were also subjects to statistical analysis. Standard deviation was calculated for the data which was obtained from the test for the repellency against *C. quinquefasciatus* mosquitoes. Each value ( $\bar{x} \pm SD$ ) represents the average of three replication.

### Phytochemical analysis of *T. indica* L. fruit extracts

The *Tamarindus indica* L. fruit extracts were screened for the presence of phytochemicals, according to the method proposed by.<sup>[15]</sup>

### Detection of alkaloids

#### Mayer's test

Extracts were dissolved individually in dilute Hydrochloric acid and treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

#### Wagner's test

Extracts were dissolved individually in dilute Hydrochloric acid and treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

#### Dragendroff's test

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

#### Hager's test

Extracts were dissolved individually in dilute Hydrochloric acid and treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

### Detection of carbohydrates

#### Molisch's test

Extracts were dissolved individually in 5 ml distilled water and filtered and the filtrates were treated with 2

drops of alcoholic  $\alpha$ -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

#### **Benedict's test**

Extracts were dissolved individually in 5 ml distilled water and filtered and the filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

#### **Fehling's test**

Extracts were dissolved individually in 5 ml distilled water and filtered and the filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

#### **Detection of glycosides**

##### **Modified Borntrager's test**

Extracts were hydrolysed with dil. HCl and were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

##### **Legal's test**

Extracts were hydrolysed with dil. HCl and were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

#### **Detection of saponins**

##### **Froth test**

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

##### **Foam test**

0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

#### **Detection of phytosterols**

##### **Salkowski's test**

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc.  $H_2SO_4$ , shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

##### **Liebermann Burchard's test**

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc.  $H_2SO_4$  was added. Formation of brown ring at the junction indicates the presence of phytosterols.

#### **Detection of phenols**

##### **Ferric chloride test**

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

#### **Detection of tannins**

##### **Gelatin test**

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

#### **Detection of flavonoids**

##### **Alkaline reagent test**

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

##### **Lead acetate test**

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

#### **Detection of proteins and aminoacids Xanthoproteic test**

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

##### **Ninhydrin test**

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

#### **Detection of diterpenes**

##### **Copper acetate test**

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

## **RESULTS AND DISCUSSION**

The solvent fractions of the fruit of *T. indica* L. is evaluated for their repellent activity against the adult of *C. quinquefasciatus* mosquito. The petroleum ether, chloroform and ethanol extracts in different concentrations were tested against three day old blood starved adult female mosquitoes to test the repellent activity. The repellent efficacy was determined in three concentrations *viz.*, 1.0, 2.5 and 5.0 mg/cm<sup>2</sup> under laboratory conditions (Table 2). The control treatment did not provide any protection even during the first trial.

#### **Evaluation of the repellent efficacy of selected fruit extracts on *C. quinquefasciatus***

All the three extracts *viz.*, petroleum ether, chloroform and ethanol showed dose dependent repellent activity. The results showed that the ethanolic extract of fruit pulp of *T. indica* L. is found to be effective against *C. quinquefasciatus* and 100% protection time was obtained

at the concentration of 5.0 mg/cm<sup>2</sup>. The repellent activity was very high at the initial stage of exposure. The increase in exposure period the repellent activity was reduced and it depends upon the concentration of the extract and density of mosquitoes.

#### Repellent activity of petroleum ether extract of *T. indica* L

Petroleum ether extract of fruit pulp of *T. indica* L. showed 93.60% protection in the first 30 minutes of exposure. 99.30% protection was obtained in the 60 minutes and 91.83% of repellency was observed in the 90 minutes at 1.0 concentration. In 2.5 concentrations, the petroleum ether extract gives 98.16% of repellency in the 30 minutes and 96.83% protection was obtained in the 60 minutes of exposure. 96% of repellency was observed at 90 minutes of extracts applied. The petroleum ether extract showed maximum repellency at 5.0 concentrations and it showed 99.33% in the first 30 minutes. Whereas 60 minutes and 90 minutes provides

more or less same repellency observed in 97% protection respectively.

#### Repellent activity of chloroform extract of *T. indica* L.

The chloroform extract of the *T. indica* L. showed 94.16% of repellency at 30 minutes of exposure and it showed 93.66% protection in the 60 minutes. 93.50% protection was observed upto 90 minutes at 1.0 concentration. 95.33% repellency was obtained for first 30 minutes at 2.5 concentrations, followed by 94.83% at 60 minutes and 92.66% at 90 minutes of exposure. In 5.0 concentrations, chloroform extract showed 95.66% protection at 30 minutes of extracts applied. 95.16% of repellency was obtained at 60 minutes and the repellency of extract was reduced to 94.50% at 90 minutes.

#### Repellent activity of ethanol extract of *T. indica* L.

Strong repellent activity and no bites were observed in the ethanolic extract of *T. indica* L. against adult *C. quinquefasciatus* and it showed 100% repellency till 90 minutes at 1.0, 2.5 and 5.0 concentrations.

**Table. 2: Repellent activity of *Tamarindus indica* L. fruit extracts against *Culex quinquefasciatus*.**

S. No	Solvent Used	Conc. Mg/ cm <sup>2</sup>	% of repellency		
			30mins	60mins	90mins
	Control	0±0	0±0	0±0	0±0
1.	Petroleum ether	1.0	93.60±0.47	93.30±0.47	91.83±0.68
		2.5	98.16±0.68	96.83±0.68	96.00±0.81
		5.0	99.33±0.94	97.83±0.68	97.16±0.89
2.	Chloroform	1.0	94.16±0.89	93.66±0.74	93.5±0.76
		2.5	95.33±0.47	94.83±0.68	92.66±0.74
		5.0	95.66±0.47	95.16±0.37	94.50±0.5
3.	Ethanol	1.0	100±0.0	100±0.0	100±0.0
		2.5	100±0.0	100±0.0	100±0.0
		5.0	100±0.0	100±0.0	100±0.0

Each value ( $\chi \pm SD$ ) represents average of three values.

#### Phytochemical screening of fruit extract of *T. indica* L.

**Qualitative analysis:** Preliminary phytochemical screening of plants part is very useful for determination of the active constituents in different solvents and their yields. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in pharmaceutical compounds

#### Phytochemical screening of fruit extract of *T. indica* L.

The petroleum ether extract of *T. indica* L. fruit revealed the presence of alkaloids, saponins, phytosterols, phenols, tannins, proteins and amino acids. The chloroform extract showed the presence of alkaloids, carbohydrates, glycosides, cardiac glycosides, phenols and diterpenes. Alkaloids, carbohydrates, glycosides, cardiac glycosides, saponins, phytosterols, tannins, phenols, flavonoids, diterpenes, proteins and amino acids were detected in the ethanolic fruit pulp extract of *T. indica* L.

**Table. 4: Phytochemical screening of fruit extracts of *Tamarindus indica* L.**

Tests	Petroleum ether	Chloroform	Ethanol
<b>Alkaloids</b>			
Mayer's Test	-	+	+
Wagner's Test	-	+	+
Dragendroff's Test	+	+	+
Hager's Test	+	+	+
<b>Carbohydrates</b>			
Molisch's Test	-	-	-
Benedict's Test	-	-	+
Fehling's Test	-	+	+
<b>Glycosides</b>			
Modified Borntrager's Test	-	+	+
<b>Legal's Test</b>	-	+	+
<b>Saponins</b>			
Froth Test	+	-	+
Foam Test	-	-	+
<b>Phytosterols</b>			
Salkowski's Test	-	-	+
Libermann Burchard's Test	+	-	+
<b>Phenols</b>			
Ferric Chloride Test	+	+	+
<b>Tannins</b>			
Gelatin Test	+	+	+
<b>Flavonoids</b>			
Alkaline Reagent Test	-	-	+
Lead acetate Test	-	-	+
<b>Proteins and aminoacids</b>			
Xanthoproteic Test	-	-	+
Ninhydrin Test	+	-	+
<b>Diterpenes</b>			
Copper acetate Test	-	+	+

(+) Detected; (-) Not detected

## DISCUSSION

### Repellent activity of fruit extract of *T. indica* L.

The transmission of mosquito-borne diseases can be interrupted by the potential insecticides of herbal origin at the individual as well as the community level.<sup>[6]</sup> Recently the natural insecticides of plant origin have been given importance due to their eco-friendly nature and biodegradability as a substitute of synthetic insecticides for the control of vectors of public health importance.

The ethanolic fruit extract of *T. indica* L. is higher repellency towards adult female *C. quinquefasciatus* mosquito than the petroleum ether and chloroform extracts. The repellent activity was found to be dose dependent and the percentage of protection was found to be directly proportional to the concentration of extract.<sup>[17]</sup> reported that the development of insecticide resistance in the population of *C. quinquefasciatus* against temephos, fenthion, cypermethrin and cyhalothrin indicating the need of search for safe, effective and alternative safe control measures.

In the present study, ethanolic extracts of leaf and fruit extract of *T. indica* L. is found to be more effective than

the petroleum ether and chloroform extract and 100% repellency was observed in lesser concentration. The study revealed that *Cymbopogon excavates* provides 100% repellency for 2 hrs against *A. arabiensis* and the repellency decreased to 59.3% after 4 hrs.<sup>[11]</sup> studied the larvicidal, repellent, and irritant potential of the seed-derived essential oil of *Apium graveolens* against dengue vector, *Aedes aegypti* L. (Diptera: Culicidae) and the results showed that the essential oil of *Apium graveolens* provides 100% repellency upto 165 minutes without causing any irritation and only one or two mosquito bites were observed until 180minutes of exposure.

The results of repellent activity of fruit extract of *T. indica* L. and leaf extract is comparable with earlier reports. In accordance to the results of the present study similar observations were reported by.<sup>[8]</sup> in which the methanol leaf extract of *Ervatamia coronaria* showed remarkable repellent properties at the higher concentration of 5.0 mg/cm<sup>2</sup> which provided 100% protection up to 150 minutes against female mosquitoes of *C. quinquefasciatus*.<sup>[5], [14]</sup> Showed that the skin repellent test at 1.0, 2.5 and 5.0 mg/cm<sup>2</sup> concentration of *Cymbopogon citratus* essential oil against the filarial mosquito *C. quinquefasciatus* showed 100% protection

upto 3 hrs, 4 hrs and 5 hrs protections respectively. The total percentage of protection of the essential oil was 49.64% at 1.0mg/cm<sup>2</sup>, 62.19% at 2.5mg/cm<sup>2</sup> and 74.03% at 5.0mg/cm<sup>2</sup> for 12 hrs.

Findings of the present study suggested that the fruit of *T. indica* L. and leaf extract explored as potential natural repellent agent and thereby protecting against the adult mosquito bites. Therefore, these plant extracts are recommended for use in the management of mosquitoes. The present investigation is an attempt to screen effective botanicals for the management of adult female *C. quinquefasciatus*. The fruit of *T. indica* L. extracts showed significant repellency against *C. quinquefasciatus* and gave protection against mosquito bites without any allergic reaction to the tested persons. The repellent activity was found to be dependent on the strength of the extracts applied.

### Phytochemical screening

#### Qualitative analysis

The phytochemical study of *T. indica* fruit petroleum ether extract revealed the presence of alkaloids, saponins, phytosterols, phenols, tannins, proteins and amino acids. Alkaloids, carbohydrates, glycosides, phenols, tannins, diterpenes were detected in the chloroform extract. Presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, proteins and amino acids were detected. Ethanolic extract of sample showed the presence of all the components such as, alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, protein and amino acids where as the di-terpenoids were found.

Plant synthesizes a wide variety of chemical compounds, which can be sorted by their chemical class, bio synthetic origin and functional groups into primary and secondary metabolites. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, because such information be of value in disclosing new resources of such chemical substances.<sup>[12]</sup> The results of the phytochemical screening of the extracts showed that the fruit pulp and leaf is rich in most of the secondary metabolites analyzed using different solvents as tabulated in Table 4 and Table 5.<sup>[13]</sup> and it has been reported that several phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens. Other compounds like saponins also have antifungal properties<sup>[1]</sup> It can therefore be assumed that the repellent activity in *T. indica* may be due to the presence of these metabolites (glycosides). Above results showed in line with the present investigation.

### CONCLUSION

The findings of this study help to assess appropriate and possible strategies to repel and control mosquito species. The fruit and leaf extract of *T. indica* L. have higher repellent efficacy against the vector *C. quinquefasciatus*.

These extracts will provide further clarity about the nature of these bioactive compounds which could become an alternative to the conventional insecticides used for repelling annoying mosquito species. However toxicity tests of the leaf and fruit extract did not caused any irritation to human skin which ascertained the safety in its usage. Hence, in the present study it could be concluded that the fruit of *T. indica* L. showed repellent activity against *C. quinquefasciatus* without any allergic reaction and it could be recommended for synthetic repellence.

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