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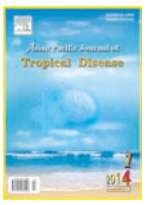

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# Phagodeterrence, larvicidal and oviposition deterrence activity of *Tragia involucrata* L. (Euphorbiaceae) root extractives against vector of lymphatic filariasis *Culex quinquefasciatus* (Diptera: Culicidae)

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PEER REVIEW

**Peer reviewer**

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**Comments**

This is a good study in which the authors have clearly shown that root extract of *T. involucrata* may be used as potent mosquitocidal agent with phagodeterrent, larvicidal and oviposition deterrent activity against *Cx. quinquefasciatus*.
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ABSTRACT

**Objective:** To evaluate phagodeterrence or biting deterrence, larvicidal and oviposition deterrence activity of *Tragia involucrata* (*T. involucrata*) root extractives against *Culex quinquefasciatus* (*Cx. quinquefasciatus*).
**Methods:** Phagodeterrence test was done using five different solvents namely petroleum ether, benzene:ethyl acetate (1:1 v/v), chloroform:methanol (1:1 v/v), acetone and absolute alcohol against the females of *Cx. quinquefasciatus*. Effective deterrence was recorded. The larvicidal bioassay was performed by solvent extractives of *T. involucrata* roots against 1st, 2nd, 3rd and 4th instars larval forms of *Cx. quinquefasciatus*. Mortality rates were recorded after 24, 48 and 72 h of exposure followed by justification of $LC_{50}$ and $LC_{90}$ values at different concentrations. Finally, the larvicidal assay was ended up by recording the health belongings of non-target water fauna. Oviposition deterrence activity of root extractives was examined against the gravid females of *Cx. quinquefasciatus*.
**Results:** About 92.85% phagodeterrence was noticed at the level of 4% of chloroform:methanol (1:1 v/v) for the very 1st hour of exposure. Entire larval populations of 1st and 2nd instars were subjected to diminution following the treatment of 0.4% and 0.5% of benzene:ethyl acetate (1:1 v/v) root extractives respectively. While 92.33% and 78.33% mortality were recorded for 3rd and 4th instars larval forms respectively. Mean effective oviposition deterrence was found to be 97.85% at 2.5% concentration level of chloroform:methanol (1:1 v/v) root extractives.
**Conclusions:** This study is a pioneer attempt to establish phagodeterrence, larvicidal and oviposition deterrence activity of *T. involucrata* root extractive.

KEYWORDS
Phagodeterrence, Larvicidal, Oviposition deterrence, *Tragia involucrata*, *Culex quinquefasciatus*

## 1. Introduction

Mosquito, living throughout the world apart from the Antarctic regions[1], is one of the most tarnished creatures in the animal kingdom with horrific reputation as highly persuasive vectors of several diseases. Altogether they cause millions of deaths annually around the globe[2]. A large proportion of the wretched victims are from the developing tropical countries. Before the onset of mosquito control programmes, mosquitos caused tens of thousands of deaths in these countries and hundreds of thousands of infections. One among such perplexing number of infection is human lymphatic filariasis, alternatively known as infectious tropical disease[3], caused by the worms

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*Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. In an approximation more than 1.2 billion people in the world are at risk of this socioeconomic crisis of lymphatic filariasis that is transmitted by female *Culex quinquefasciatus* (*Cx. quinquefasciatus*) mosquito from man to man[4]. *Wuchereria bancrofti*, exploiting human body as their definitive host for survival and efficiently transmitted by the vector *Cx. quinquefasciatus*, is the principal causative organism of human lymphatic filariasis in Indian subcontinent accounting for roughly one third of total case numbers in the world. Thus, to minimize the incidence of this disease, control of mosquito population is very essential which will subsequently reduce the fatality of several diseases by reducing disease burden. Use of ecofriendly insecticides derived and purified from biological origin, mostly from plants, are best advised nowadays for their selective toxicity and non-residual effects in contrast to their synthetic counterparts[5]. Though the propensity of insecticides resistance can be lower down by judicious and scientific measures, being an evolutionary phenomenon, it can not be avoided solely. A number of genetic resistance cases against synthetic pesticides and more shockingly cases of biopesticide resistance have made it more complicated to rely on accessible mosquitocidal agents exclusively for long term applications[6,7]. So the findings of new organochemicals are obvious in a regular fashion and, from the dawn of civilization, plant kingdom nearly suffices the need. The Euphorbiaceous flowering plant genus, *Tragia*, comprises of nearly hundred species distributed throughout the tropical and subtropical part of the world. Plant extractives of *Tragia involucrata* (*T. involucrata*) have been used as ethnomedicine since prehistoric times and in India their use in "Sidhha" medicine by Tamil people is wide spread. *T. involucrata* root extractives are proven to act as potent antimicrobial compounds and a useful fungicide as well[8]. As per our literature survey was concerned, no information was available on the mosquitocidal activities of *T. involucrata* root extractives.

Aiming at extracting and developing an efficient mosquito control agent, the present study was carried out to evaluate phagodeterrence or biting deterrence, larvicidal and oviposition deterrence activity of *T. involucrata* root extractives against *Cx. quinquefasciatus*.

## 2. Materials and methods

### 2.1. Collection of plant material

Several noseburn populations are speckled along the greenery borders of the Golapbag campus, University of Burdwan. Roots were selectively unruffled during the August and September, 2012 from mature plants and the voucher specimen is deposited in the herbarium of the Department of Zoology (voucher No. GCK-04), University of Burdwan, West Bengal, India.

### 2.2. Preparation of different solvent extracts

Solvent extraction was carried out by tissue homogenization accompanied by maceration both of which pertain to cold extract procurements at room temperature[9,10]. Plant tissue homogenization in solvent has been widely used by researchers. In the present assessment, a combined protocol of Das *et al.* and Ncube *et al.* was followed with very little modification[9,10]. The dried roots of 25 g weight were grinded in a blender to fine particles, put in a 250 mL of solvent and shaken vigorously for 5-10 min and left for 24 h after which the extract was filtered through Whatman No. 1 filter paper. The filtrates were, then, dried and redissolved in the solvent to determine the concentration (in percentage). Further, crudely powdered cleaned dried roots (25 g each time) of *T. involucrata* were decocted with five different solvents namely petroleum ether, benzene:ethyl acetate (1:1 v/v), chloroform:methanol (1:1 v/v), acetone and absolute alcohol in stopper containers (250 mL every time) for a period of 3 weeks (21 d) with frequent agitation (158 $min^{-1}$) in an automated shaker. Finally, stock solution for a particular solvent extract was prepared by mixing the solvent extractives isolated in two different procedures (1:1 v/v) and the processes were repeated five times to acquire all the stocks for five different types of solvents. Each derived solvent extract was intensified by evaporation in rotary evaporator. Working concentration gradients were prepared ranging from 0.1% (w/v) to 4.0% (w/v) by the solid residues of each evaporated solvent extracts with mixing of determined amount of double distilled water.

### 2.3. Mosquito culture

Egg strips of *Cx. quinquefasciatus* were assembled with acute precision from some drains contiguous to University campus (23.16°N, 87.54°E) and reared in the Mosquito and Microbiology Research Units, Department of Zoology, University of Burdwan, West Bengal, India, to set up the colony. Following the protocol of Sharma and Saxena[11], with tiny variations, colonies were maintained in insectary (45 cm×30 cm×10 cm) at (27±1) °C and (80±2)% relative humidity with a photoperiod of 13:11 hour light and dark cycles respectively[10]. Dechlorinated tap water was used for the purpose of hatching. Larvae were fed with the supplementary diet of delicately ground brewer yeast and dog biscuits (3:1) and laboratory conditioning of the reared larvae were practiced for further assessments. The transformed pupae were alienated manually with a glass dropper inside a glass beaker (500 mL) filled with tap water. For the purpose of adult emergence of mosquitoes, the beaker was introduced into cages. A cotton ball soaked in 10% glucose solution and 10% multivitamin syrup was used for providing meal to the adult mosquitoes which were periodically feed on

immobilized chick blood following the protocol of Reuben with little modification[12].

### 2.4. Phagodeterrence test

Biting deterrency of adult *Cx. quinquefasciatus* was evaluated with all the different solvent extractives having myself as exclusive foundation of human volunteer. The test was performed in between 5.30 pm to 8.30 pm, as the focal dusk time was in and around 5.45 pm in the stipulated months, in the mosquito research laboratory of the Department of Zoology, University of Burdwan. The arms were covered with rubber sleeve having a window (3 cm× 10 cm) in the ventral surface after thorough cleaning with double distilled water. In a wooden cage (30 cm×30 cm×30 cm) which was roofed with mosquito net, 250 pupae were introduced 4 d before the commencement of the test. The wooden cage was devised to supply 10% glucose solution as food source to the newly immerged adult mosquitoes. Now the food source was removed for 24 h prior to testing. The treated arm *i.e.* hand smeared with 2 mL solvent extract of *T. involucrata* at the window position along with the control arm *i.e.* hand treated with 2 mL chloroform alone at the same position were inserted into the cage simultaneously for 10 min at each turn, and no of turns were repeated up to 3 h with an interval of 30 min at the completion of each turn. The working concentrations of each solvent extract was subjected to several trials, and for obtaining good quality phagodeterrent activity, the graded concentrations were raised up to 4% starting from 2.5%. The degree of phagodeterrence was calculated as protection percentage following the standard protocol of Ansari *et al.*[13]:

Protection percentage (%)= (C−T)/C

Where C and T represent the no of bites in the control and treated arm respectively.

### 2.5. Dose-response larvicidal bioassay: demeanor of wrigglers with extractives

Treated the WHO protocol as standard[14], the larvicidal bioassay was performed, with slight modifications, at the Mosquito Research Laboratory, University of Burdwan. All instars of *Cx. quinquefasciatus* larvae were tested against all the extractive gradients (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) which were prepared earlier. Each experiment was carried out in triplicate with a set of controls filled with distilled water and without any extractive. Twenty larvae of specific instar stage were placed in Petri dishes of 9 cm diameter (150 mL capacity), filled with 100 mL of tap water very selectively and this processes were repeated for all sorts of combinations taking different concentration gradients (0.1% to 0.5%) and instars (first to fourth) together. After devising the initial arrangement, each Petri dish was kept at room temperature (27±1) °C, within (80±2)% relative humidity range for an observation period of 72 h in totality. The larvae were considered as dead when they failed to exhibit any movement after being pricked by a sharp needle in the siphon or cervical region or they were incapable to accomplish the water surface[15]. The number of dead wrigglers was counted every 24 h interval upto 72 h and percentage mortality was recorded from the mean average value of three replicates. The mortality data of 48 h and 72 h were articulated by adding up the mortality of 24 h and 48 h, respectively.

### 2.6. Assay of oviposition deterrence

An evaluation on oviposition deterrence activity of *T. involucrata* root extractives was performed using the standard protocols of Rajkumar and Jebanesan and Elimam *et al.* with little modifications[16,17]. Three clean cages (A, B and C) were needed to make germ free for this experiment. In each cage, a good number (20) of well fed (by immobilized chicken blood) gravid females were unconstrained for laying purpose. Glucose soaked cotton balls were kept in each cage for associative feeding occasions. A somewhat lower concentration of solvent extracts *i.e.* 1.5% was kept inside cage A, cage B equipped with intermediate concentration of extract *i.e.* 2.0% and cage C contained one higher concentration of extract *i.e.* 2.5%. Each control container contained 100 mL distilled water and treated containers contained 100 mL distilled water along with the determined amount of solvent extracts. Every sort of experiment was performed thrice (control a, control b and control c and treated a, treated b and treated c, where a, b and c are replicates only). At the end of 48 h, number of laid eggs were counted in treated and control containers separately inside the cage. Rising the formula of Elimam *et al.*[17], effective oviposition deterrence was calculated as follows:

Effective Deterrence (%)= [(NC−NT)/NC]×100

Where NC=number of eggs laid in control, NT= number of eggs laid in treated solution.

### 2.7. Effect on non-target organisms

The minute creatures sharing the same ecological micro-environment are considered under the most fatal non targeted risk group. Susceptibility of these organisms to the root extractive was carried out by using *Chironomus circumdatus* larvae (insect) as representative non-targeted population. They were exposed to root extractives at the concentration level of $LC_{50}$ at 24 h of 3rd instars larvae to scrutinize the mortality and other abnormalities such as slowness of swimming activity up to 72 h of exposure.

### 2.8. Phytochemical analysis of the plant extract

Following the methodology of Harbone[18] and Stahl[19], a brief phytochemical study of the root extractives was carried out.

### 2.9. Statistical interpretation

Using Abbott's formula[20], the percentage of corrected mortality was considered. By virtue of certain statistical software such as "Stat Plus 2007 (Trial Version)" and MS Excel 2002, several statistical aspects were figured out including $LC_{50}$, $LC_{90}$, regression equations ($Y$=mortality, $X$=concentrations), regression coefficients, and completely randomized three-way ANOVA. The statistical significance of differences between solvent extracts was determined by Student's $t$-test where significance level was set at 5%.

## 3. Results

Several trials prominently established the phagodeterrent or feeding deterrent activity of chloroform:methanol (1:1 v/v) extractives of *T. involucrata* which was significantly ($P$<0.05) higher than that of petroleum ether ($t$=11.342), benzene:ethyl acetate ($t$=9.401), acetone ($t$=5.092) and absolute alcohol ($t$=4.253) (against tabulated value of 2.17). Using graded concentrations, it was evaluated that maximum phago-deterrence was achieved for the very 1st spell of 3 h of exposure and at the concentration level 4.0% which exhibited 92.85%, 77.08%, 72.60% of protection percentage for the very 1st , 2nd and 3rd hours of exposures. However, after the very 3rd hour of exposure, there were a stiff decrease in protection percentage and those data were excluded. Every possible type of combinatorial data was given by the graphical representation of protection percentage in Figure 1.

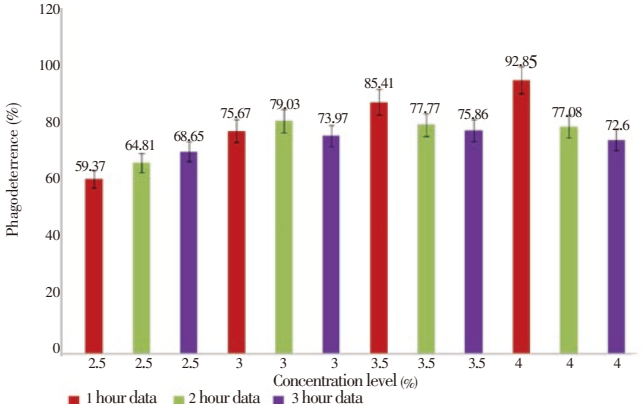


**Figure 1.** Graphical representation (with 5% error bar) of phagodeterrence of chloroform: methanol (1:1 v/v) extractive of *T. involucrata* roots against *Cx. quinquefasciatus*.

Benzene:ethyl acetate (1:1) was proven to be efficient solvent combination, comparatively, for extraction of mosquito larvicide from *T. involucrata* roots when tested under laboratory condition exhibiting significantly higher ($P$<0.05) larvicidal activity than petroleum ether ($t$=10.419) and absolute alcohol ($t$=8.087). Moreover, no larval death was recorded in the case of both chloroform:methanol (1:1 v/v) and acetone. The 1st instars were killed entirely (cent percent mortality) after 48 h of exposure with 0.4% of root extractive solution (Table 1), while cent percent mortality of treated 2nd instars group occurred at 72 h of exposure at concentration level 0.5% when exposed to benzene:ethyl acetate (1:1 v/v) root extractive under laboratory condition. Significantly high amount of mortality were noticed in case of 3rd and 4th instars of *Cx. quinquefasciatus* mosquito at concentration level 0.5% and 72 h of exposure period (91.33% and 78.33%) though no cent percent mortality resulted in any trial. An increase in $LC_{50}$ and $LC_{90}$ values (at 95% confidence level) regarding late stage larvicidal effects were noticed, through Log probit analysis and regression analysis (Table 2) which was noticed to low down with increase in exposure period.

**Table 1**
Dose response larvicidal assay using benzene:ethyl acetate (1:1 v/v) extractives of *T. involucrata* root against *Cx. quinquefasciatus*.

| Larval Instars | Concentrations (%) | Mortality rate 24 h | 48 h | 72 h |
|---|---|---|---|---|
| First | 0.1 | 16.67±0.39 | 39.55±0.27 | 66.67±0.58 |
| | 0.2 | 26.66±0.37 | 56.22±0.21 | 82.44±0.00 |
| | 0.3 | 43.33±0.31 | 67.88±0.17 | 88.11±0.66 |
| | 0.4 | 96.660±0.023 | 100.00±0.00 | 100.00±0.00 |
| | 0.5 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 |
| Second | 0.1 | 13.33±0.37 | 27.22±0.00 | 32.44±0.88 |
| | 0.2 | 23.85±0.35 | 47.11±0.66 | 58.57±0.68 |
| | 0.3 | 44.26±0.29 | 52.28±0.57 | 63.33±0.88 |
| | 0.4 | 93.33±0.05 | 96.33±0.88 | 98.67±0.05 |
| | 0.5 | 96.67±0.05 | 98.47±0.66 | 100.00±0.57 |
| Third | 0.1 | 6.67±0.27 | 14.44±0.00 | 33.33±0.68 |
| | 0.2 | 16.66±0.25 | 28.66±0.33 | 42.22±0.68 |
| | 0.3 | 33.33±0.20 | 40.67±0.68 | 55.47±0.88 |
| | 0.4 | 43.47±0.18 | 57.00±0.66 | 68.89±0.33 |
| | 0.5 | 83.21±0.04 | 88.66±0.88 | 91.33±0.68 |
| Fourth | 0.1 | 3.33±0.14 | 8.66±0.58 | 16.22±0.00 |
| | 0.2 | 10.00±0.13 | 28.55±0.68 | 39.44±0.58 |
| | 0.3 | 16.67±0.10 | 32.33±0.33 | 41.55±0.57 |
| | 0.4 | 30.35±0.08 | 38.67±0.00 | 53.66±0.88 |
| | 0.5 | 40.42±0.08 | 56.66±0.57 | 78.33±0.33 |

Data are expressed as mean±SE.

**Table 2**
Determination of $LC_{50}$ and $LC_{90}$ through log-probit and regression analyses.

| Larval Instars | Period of exposure (h) | $LC_{50}$ | $LC_{90}$ | Regression | $R^2$ |
|---|---|---|---|---|---|
| 1st | 24 | 0.26 | 0.48 | $23.33X-1.40$ | 0.95 |
| | 48 | 0.15 | 0.38 | $16.78X+2.28$ | 0.93 |
| | 72 | 0.07 | 0.24 | $8.33X+6.23$ | 0.88 |
| 2nd | 24 | 0.25 | 0.52 | $23.67X-1.70$ | 0.93 |
| | 48 | 0.19 | 0.47 | $18.83X+0.83$ | 0.92 |
| | 72 | 0.16 | 0.40 | $17.00X+1.97$ | 0.94 |
| 3rd | 24 | 0.36 | 0.88 | $18.33X-1.77$ | 0.95 |
| | 48 | 0.30 | 0.85 | $17.77X-0.83$ | 0.96 |
| | 72 | 0.20 | 0.84 | $14.06X+1.56$ | 0.91 |
| 4th | 24 | 0.68 | 2.67 | $9.33X-0.80$ | 0.88 |
| | 48 | 0.49 | 2.63 | $10.33X+0.30$ | 0.87 |
| | 72 | 0.30 | 1.13 | $14.00X+0.47$ | 0.92 |

$X$: concentration of solvent extracts.

The larvicidal effect of *T. involucrata* root extractive against *Cx. quinquefasciatus* at 72 h of exposure were found to be statistically significant ($P<0.05$) when the mortality rate ($Y$) was correlated positively with the concentration of exposure ($X$) having regression coefficient ($R^2$) close to 1 in each case and through ANOVA analyses (Table 3). However, the non-target populations were remained nonresponsive when treated with root extractives at the $LC_{50}$ level of 3rd instars for a period of 72 h. Oviposition deterrent effect of *T. involucrata* root was found using chloroform:methanol (1: 1 v/v) against gravid females of *Cx. quinquefasciatus*. Plotting the number of eggs laid in control and treated trials separately over $Y$ axis and concentrations on $X$ axis a graph was prepared (Figure 2) that entailed the oviposition deterrence activity of *T. involucrata* root extractives using chloroform:methanol (1:1 v/v) as solvent.

**Table 3**

Completely randomized three way ANOVA analysis using concentration, hour and instars as three parameters.

| Source of variation | Sum of squares | Degree of freedom | Mean of squares | $F$ value | $P$ level |
|---|---|---|---|---|---|
| I | 395.39 | 3 | 131.79 | 213.73 | 0 |
| H | 170.43 | 2 | 85.22 | 138.19 | 0 |
| C | 931.58 | 4 | 232.89 | 377.67 | 0 |
| I×H | 10.99 | 6 | 1.83 | 2.97 | 0.0097 |
| I×C | 74.24 | 12 | 6.19 | 10.03 | 0 |
| H×C | 27.12 | 8 | 3.39 | 5.49 | 0 |
| I×H×C | 36.79 | 24 | 1.53 | 2.48 | 0.0006 |
| Residual | 74.00 | 120 | 0.62 | – | – |
| Total | 1720.55 | 179 | 9.61 | – | – |

C: concentration; H: hour; I: instars.

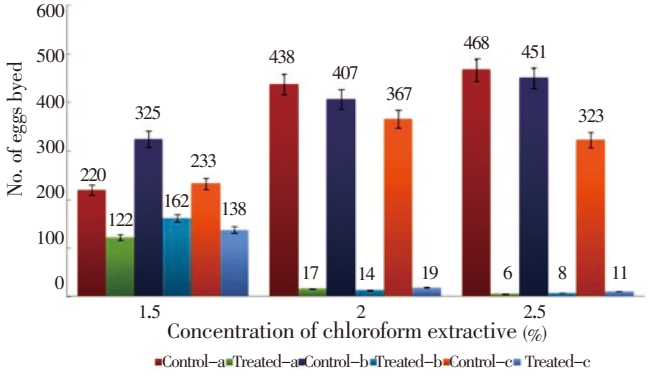


**Figure 2.** Graphical representation of oviposition in control and treated [chloroform:methanol (1:1 v/v) extractive of *T. involucrata* roots] trials with error bar (5%).

Mean effective oviposition deterrence was found to be 97.85% at 2.5% concentration level of root extractives. Several plant secondary metabolites were found to be present in usual or conjugated forms including saponins, terpenoids, alkaloids, steroids, tannin, flavonoids, glycosides and free glycoside bound anthroquinones *etc*.

## 4. Discussion

Reducing mosquito borne diseases remains a big challenge even at the most advancement of modern sciences. Personal protection from mosquito bites through several synthetic and herbal products are often practiced. But synthetics are mostly non biodegradable, having harmful residual hazards as well as costly[21].

Feeding deterrence activity of this medically important sporadic pest by means of photochemical is of utmost importance as deterrence can reduce the mosquito borne diseases by reducing the biting incidences resulting in low transmission rate of diseases. Phagodeterrency of several herbal essential oils against *Aedes aegypti* and *Anopheles dirus* was evaluated earlier[22]. Biting deterrence of *Eucalyptus* and *Azadirachta indica* seed oil on *Cx. quinquefasciatus* was proved to be very effective[23]. Prabhu *et al.* noticed 90.41% biting deterrency at 100% concentration of *Moringa olifera* that was subjected to reduction upto 23.28% when treated with 20% concentration[24]. Root extractives of indigenous plant *Echinochloa stagnium* using petroleum ether as extraction media evoked 100% biting deterrence or phagodeterrence when applied at a dosage of 4.3 mg/ $cm^2$[25]. The present study revealed elevated phagodeterrence activity (92.85%) of *Tragia involucrata* root extractives at the level of 4% concentration for a period of 3 h against the females of *Cx. quinquefasciatus* using chloroform:methanol (1:1 v/v) as extraction media.

Confinement to water bodies and very low rate of dispersal make the mosquito larvae most vulnerable and hence larval control is the best strategy to reduce mosquito population at very early stage. Researchers exposed ecofriendly mosquito larvicidal potentiality of several plants[26-28]. Generally polar fractions of plant materials are efficiently extracted using polar solvents and non polar solvents take out non polar molecules. Essential oils are commonly extracted by nonpolar solvents such as petroleum ether and hexane (polarity index of 0.1)[29]. Comparatively, moderate polar solvents like chloroform (polarity index of 4.1) mainly pull out steroids[30], alkaloids[31] *etc.* from botanicals. Govindarajan reported that methanol extract of *Andrographis paniculata, Eclipta alba*, and *Cardiospermum halicacabum* are very much effective against the larvae of *Anopheles stephensi* ($LC_{50}$=79.68, 112.56, and 133.01 mg/L; $LC_{90}$=154.66, 220.68, and 270.72 mg/L, respectively)[32]. Ireri *et al.* evaluated the efficacy of *Tagetes minuta* (Asteraceae) (*T. minuta*), *Acalypha fruticosa* (Euphorbiaciae) (*A. fruticosa*) and *Tarchonanthus camphoratus* (Compositae) extracts for the control of *Phlebotomus duboscqi*[33]. The extracts showed significant mortality ($P<0.05$) to both males and females and had comparable low $LD_{50}$ values in *T. minuta* and

*A. fruticosa* extracts bioassays. The lowest $LD_{50}$ value for females was 10.6 mg/mL using ethyl acetate extractives of *T. minuta*, while the highest was 12.0 mg/mL for methanol extractive. Males had the lowest value of 9.9 mg/mL in *T. minuta* methanol extract, while the highest was in *A. fruticosa* methanol extract with a value of 15.5 mg/mL. Results however showed that there was no significant mortality ($P>0.05$) difference between males and females but mortality significantly differed ($P<0.05$) at various concentrations[33]. Present study exposed the successful use of benzene:ethyl acetate (1:1 v/v) root extractives of noseburn to kill cent percent *Cx. quinquefasciatus* at 1st and 2nd instars stages and considerably high amount of mortality against 3rd and 4th instars of wrigglers (92.33% and 78.33% respectively) following an exposure period of 72 h under laboratory condition.

Reducing the mosquito breeding ground through use of oviposition deterrent is another way to fight against mosquito populations. Oviposition deterrent effects of selected essential oils against *Anopheles stephensi, Aedes aegypti* and *Cx. quinquefasciatus* were brought into front by Prajapati *et al*[34]. The current study revealed appreciably high amount (97.85%) of effective oviposition deterrence in relatively moderate concentrations (2.5%) of chloroform:methanol (1:1 v/v) root extractive.

Root extractives of *T. involucrata* offer a potent mosquitocidal agent having phagodeterrent, larvicidal and oviposition deterrent activity of admirable quality at a time against *Cx. quinquefasciatus*. The consequence of present study may lead to the development of new mosquitocidal of plant origin through extensive research and help in the mosquito control measures.

## Conflict of interest statement

We proclaim that we have no divergence of interest.

## Acknowledgements

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## Comments

### *Background*

Mosquito borne disease becomes a major problem. Herbal products are recommended for vector control. In order to combat mosquito borne diseases, alternatives to pesticides are searched. The present study has been able to demonstrate this aspect by using root extract of *T. involucrata* against *Cx. quinquefasciatus*.

### *Research frontiers*

The present study has clearly established phagodeterrence, larvicidal and oviposition deterrence activity of *T. involucrata* root extract against *Cx. quinquefasciatus*.

### *Related reports*

Root extract of *T. involucrata* offer a potent mosquitocidal agent having phagodeterrent, larvicidal and oviposition deterrent activity of admirable quality at a time against *Cx. quinquefasciatus*. Present study has confirmed these findings.

### *Innovations & breakthroughs*

This paper clearly indicated that resistance of mosquito against insecticide and pesticide is an evolutionary phenomenon; entails searching of new botanicals is very much necessary and root extract of *T. involucrata* offers a potent mosquitocidal agent.

### *Applications*

This type of study has huge importance where proper herbal alternatives may be searched for mosquito control.

### *Peer review*

This is a good study in which the authors have clearly shown that root extract of *T. involucrata* may be used as potent mosquitocidal agent with phagodeterrent, larvicidal and oviposition deterrent activity against *Cx. quinquefasciatus*.

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