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# LARVICIDAL EFFECTS OF ETHANOLIC EXTRACT OF FLOWERS (BUDS AND CALYX) OF *TAGETES ERECTA* AND ITS CHLOROFORM AND PETROLEUM ETHER SOLUBLE FRACTIONS AGAINST THE LARVAE OF *AEDES AEGYPTI*

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## ABSTRACT

The mosquito-borne diseases, dengue fever, malaria, encephalitis, yellow fever, chikungunya, filariasis, are causing havoc in many countries, and loss in terms of human lives is irreversible. *Aedes aegypti* the primary vector for dengue fever, dengue haemorrhagic fever and yellow fever is widespread over large areas of the tropics and subtropics; and is reported to infect more than 100 million people every year in more than 110 countries in the tropics. Application of chemical insecticides, though undesirable, is still the major tool adopted globally for mosquito control but has reflected in various drawbacks. In the current era, research is focused on natural products to combat these disease transmitting vectors and a recent emphasis has been placed on plant material. Plants enriched with phytochemicals are reported to possess insecticidal properties particularly larvicidal**.** In the present study larvicidal activity of ethanol extract, petroleum ether and chloroform fractions of buds and calyx of *Tagetes erecta* was tested against *Aedes aegypti*. The fresh flowers of *T. erecta* were extracted in cold with ethanol and after concentration, the ethanol extract was fractionated with chloroform and petroleum ether to afford a brownish syrupy suspension of ethanol extract petroleum ether soluble fraction and chloroform soluble fraction. The larvicidal effect of ethanol extract and their solvent fractions were determined by the standard procedure of WHO against different instars of *Aedes aegypti*. The larval mortality rate of *T. erecta* was entirely time and dose dependent. It can be concluded that the flowers of *T. erecta* are very effective natural larvicide and could be useful against *Aedes aegypti.*

**KEYWORDS:** *Tagetes erecta*, *Aedes aegypti,* Larvicidal effect.

## 1. INTRODUCTION

Insect-transmitted disease remains a major source of illness and death. Mosquitoes are responsible for the transmission of many medically important pathogens and parasites such as viruses, bacteria, protozoans and nematodes, which cause serious diseases such as malaria, dengue, yellow fever, Chikungunya fever and filariasis (Kettle, 1995). *Aedes aegypti* is one of the mosquito species responsible for the transmission of vector borne diseases (Kovendan and Murugan, 2011). World Health Organization (WHO) stated that about 2/5 of the global human population are currently threaten of dengue and the best way to control the transmission of dengue virus is fight the mosquitoes that cause the disease. *Aedes aegypti* is a tropical mosquito, found in greatest profusion in Central Africa, from where it is originated (Gubler, 1997). Dengue infection starts with simple fever that leads to severe and sometimes fatal dengue haemorrhagic fever (DHF) the other type is dengue shock syndrome (DSS) (WHO, 2009). Symptoms of usual simple dengue usually start with fever within 4 to 7 days after bitten by an infected mosquito. Through the bites of infective female *Aedes aegypti* mosquitoes, Dengue viruses are transmitted to the vulnerable human host (Raja *et al*., 2009). Due to the pathogenic diseases and serious harms caused by mosquitoes, controlling them has been the primary subject of several new researches over the past few years. Mosquito control includes targeting the adult mosquito through spraying chemical insecticides or by killing the mosquito larvae before they emerge into adults via using synthetic larvicides (Invest, 2008; Tiwary *et al*., 2007). Mosquitoes have been developed resistance against the chemical insecticides. Chemical insecticides greatly affect the living beings and environment as well. There is an imperative demand to search novel eco-friendly substitutes which are most active and cost effective than the synthetic insecticides. Plants have a great diversity of naturally occurring biologically potent chemicals that can be used as larvicidal agents, plant based biochemicals have wide range activity against target species and are considered safe for environment (Wattal *et al*., 1981; Sukumar *et al*., 1991). Phytochemicals obtained from the huge diversity of plant species are important source for safe and biodegradable chemicals, which can be screened for mosquito repellent, larvicidal, and insecticidal activities; and tested for mammalian toxicity. Plant based products does not have any hazardous effect on ecosystem. Recent research has proved that effectiveness of plant derived compounds, such as saponin, steroids, isoflavonoids, essential oils, alkaloids and tannins has potential mosquito larvicides (Wiseman *et al*., 2005; Chowdry *et al*., 2008; Ghosh *et al*., 2008) Plant secondary metabolites and their synthetic derivatives provide alternative source in the control of mosquitos (Yang *et al*., 2004). Therefore, researchers are currently exploiting natural substances to be used as insecticides for controlling larval mosquitoes (Moretti Mario *et al*., 2002; Cetin *et al*., 2004).

*Tagetes erecta* a common aromatic herb and a popular stout annual garden herbaceous plant belongs to the family Asteraceae. It is originated in North and South America and are widely cultivated in other Asian countries viz., Bhutan, China, Nepal and IndiaIt is known as 'marigold' in English, 'Thulukka samanthi' in Tamil and 'genda' in Hindi. *Tagetes erecta* is a medicinal plant which has a high therapeutic value in the field of medicine.The plant possesses phytochemical constituents viz., thiophenes, flavonoids, carotenoids, triterpenoids, glycosides, terpenoids, alkaloids, quinines, phenols, coumarins, carbohydrates, tannins, steroids, terpenes and salicylic acid. Some of the phytocompounds extracted from *Tagetes erecta* include quercetagetin, syringic acid, methyl-3, 5-dihydroxy-4- methoxy benzoate, quercetin, thienyl, ethyl gallate, piperitone and D-limonene. Further, the plant is used as a poison for invertebrates and in plant pest control. The plant possesses antibacterial, antimicrobial, antioxidant, analgesic, nematicidal, wound healing and hepatoprotective property. The plant is reported to possess insecticidal activity against *Tribolium castaneum* and mosquito species viz., *Anopheles stephensi* and *Culex quinquefasciatus* (Murugesan Sakthivadivel *et al*., 2016; Nikkon *et al*., 2009; Green *et al*., 1991).Hence the present investigation is attempted to study the larvicidal activity of ethanol extract, petroleum ether and chloroform fractions of buds and calyx of *Tagetes erecta* against *Aedes aegypti*

## 2. MATERIALS AND METHODS

### 2.1. Collection of Plant materials

Fresh flowers of *Tagetes erecta* were collected from the Kaivalya Botanical garden, Anna nagar, Chennai (collection date 10.03.2016) has been deposited. Flowers were authenticated by the Post graduate and research department of Botany of Pachaiyappas's College, Chennai, Tamil Nadu, India.

### 2.2. Extraction and fractionation

The fresh flowers of *T. erecta* were dried for 14 days and finally dried in an oven below 80 °C. Buds and calyx were separated from *T. erecta* flowers. Plant materials (500g) were then extracted in cold with ethanol (2.5. L). After concentration, the ethanol extract was fractionated with chloroform and petroleum ether. The solvents were concentrated by rotary evaporator at 40 °C under reduced pressure to afford a brownish syrupy suspension of ethanol extract (50.0 g), petroleum ether soluble fraction (18.6 g) and chloroform soluble fraction (23.8 g).

### 2.3. TLC screening

All extracts were run on pre-coated silica gel plate using petroleum ether and ethyl acetate (9:1 and 7:5) as the mobile phase and vanillin-$H_2SO_4$ reagent was used as spray reagent. Ethanol extract of flower gave positive test for glycosides but the chloroform and petroleum ether soluble fractions mainly showed the presence of terpenoids and flavonoids (Harborne, 1984).

### 2.4. Test insects

Larvae of the test mosquito were reared at (27±1) °C, 40%-60% relative humidity and a 12:12 h light: dark photoperiod in laboratory. To rear larvae for toxicity assay, single egg rafts were placed in a number of 600 mL glass beakers containing 450 mL distilled water. The larvae were fed with powdered Brewer's yeast at 10, 20, 40 and 80 mg per beaker everyday for first, second, third and fourth instars larvae, respectively. Water was changed every day to avoid scum formation, which might create toxicity.

### 2.5. Larvicidal bioassay

The larvicidal effect of crude ethanol extracts and their solvent fractions were determined by the standard procedure of World Health Organization (WHO, 1996). The stock solutions were prepared by dissolving extracts and fractions (10 mg of each) in 1 mL of dimethyl sulphoxide (DMSO). After that thirty laboratory reared first, second, third and fourth instars larvae were released into 100 mL glass beakers separately, containing 50 mL of distilled water to which 50, 100, 200 and 400 µL of each stock solutions were added using capillary micro-pipettes to get the desired test concentrations (w/v), *viz*, 10, 20, 40 and 80 µg/mL. Three types of control were maintained: i) distilled water; ii) distilled water plus food medium and iii) distilled water plus solvent (DMSO). Three replicates were made for each concentration and the experiment was performed under laboratory conditions at (27±1) °C and 40%-60 % relative humidity. Brewer's yeast was supplied as a larval food during the test periods for larval feeding.

### 2.6. Statistical analysis

The mortality data were then subjected to Probit analysis for the determination of $LC_{50}$ values using the computer software SPSS of 14 version. Results with $P<0.05$ were considered to be statistically significant.

## 3. RESULTS

The crude ethanol extract of flowers of *T. erecta* and chloroform and petroleum ether soluble fractions were highly effective against the first, second, third and fourth instars larvae of *Aedes aegypti* (Table 1). The chloroform soluble fractions showed the highest toxicity than the other samples and consequently, the lowest $LC_{50}$ values (14.14 µg/mL, 17.06 µg/mL, 36.88 µg/mL and 75.48 µg/mL) in all instar larvae. The larvae showed comparative tolerance with the increase of their age and time, and $LC_{50}$ values of all samples increased in all the instars tested (Table 1). The lowest $LC_{50}$value for ethanol extract was found to be 113.91, 113.14, 256.67 and 1023.04 µg/mLin first, second, third and fourth instar larvae after 48 h of exposure, whereas petroleum ether fraction had lowest $LC_{50}$ (59.55 µg/mL) value against first instar larvae after 24 h of exposure($P<0.05$) (Table 1).

The increase in mortality during the course of exposure period could be due to several factors, which may be acting separately or jointly. For example, the uptake of the active moiety of the compound could be time dependent, leading to a progressive increase in the titer of the plant-derived compounds tested and its effect on the larval body, the active moiety of the compound could get converted into more toxic metabolites in the larval integument and alimentary canal, resulting in time-dependent effects.

*T. erecta* extracts possessed larvicidal activity against *Aedes aegypti* and larvae of *Meloidogyne incognita* (Natarajana *et al*., 2006; Pavela, 2002; Elango *et al*., 2009).

Nikkon *et al*. (2009) reported that the crude ethanolic extract of *Tagetes erecta* flowers possessed larvicidal activity against the larvae of *Culex quinquefasciatus*. Similarly, Beena Joshi Bhatt (2015) opined the essential oil of *Tagetes erecta*possesed the larvicidal activity aganist*Aedes aegypti*. *Tagetes erecta* extracts caused mortality to *Aedes agypti* larvae which can also be attributed to the presence of terpenoids, an insecticidal compound. Reduction in the rate of mortality could be due to biodegradation of terpene in water as its resistance in water is reduced by its volatilization (Raymond, 1995). All these reports emphasize that *Tagetes erecta* extracts possess lethal effects against *Aedes agypti*. Many plant species have been screened for their larvicidal activity against *Aedes aegypti* in the recent years. The successful results of the present study on mosquitocidal potential of crude ethanol extract, chloroform and petroleum ether soluble fractions of flowers of *T. erecta* encourage further efforts to investigate the bioactive compounds that might possess good larvicidal properties. Tehri and Singh (2015) stressed that the activity of botanicals is generally attributed to some particular compounds but if a synergistic phenomenon is established among these metabolites it may result in an increased bioactivity compared to isolated components, thus enhancing the effectiveness. The future potential use of extracts from genus *Tagetes* as botanical insecticides will require their phytochemical analysis and examination of insecticidal activity of the individual biochemically characterized components.

**Table 1: Mosquitocidal activity of flower of *T. erecta* against *A. aegypti* larvae.**

| Plant extracts | Larval stage | Exposure time (h) | $LC_{50}$ (μg/mL) | *Chi*-squre value ($\chi^2$) |
|---|---|---|---|---|
| Ethanol extract | 1st instar | 12 | 330.89 | 0.175 7* |
| | | 24 | 202.67 | 0.206 4* |
| | | 48 | 113.91 | 0.235 8* |
| | 2nd instar | 24 | 320.63 | 0.001 0 |
| | | 48 | 113.14 | 0.010 3 |
| | 3rd instar | 24 | 918.62 | 0.033 5 |
| | | 48 | 256.67 | 0.037 1 |
| | 4th instar | 24 | 3783.33 | 0.089 8 |
| | | 48 | 1023.04 | 0.032 3 |
| Petroleum ether fraction | 1st instar | 6 | 121.08 | 0.388 3 |
| | | 12 | 74.19 | 0.830 4 |
| | | 24 | 59.55 | 2.170 1* |
| | 2nd instar | 12 | 167.89 | 0.123 5 |
| | | 24 | 114.64 | 0.154 0 |
| | | 48 | 77.24 | 0.302 1* |
| | 3rd instar | 24 | 403.58 | 0.199 1 |
| | | 48 | 142.52 | 0.122 5* |
| | 4th instar | 24 | 829.90 | 0.227 4 |
| | | 48 | 386.42 | 0.331 8 |
| Chloroform fraction | 1st instar | 3 | 28.59 | 0.675 9* |
| | | 6 | 19.95 | 0.744 2* |
| | | 12 | 14.14 | 1.533 0* |
| | 2nd instar | 6 | 37.72 | 0.326 8* |
| | | 12 | 24.71 | 0.277 2* |
| | | 24 | 17.06 | 1.936 5* |

| | | | | |
|---|---|---|---|---|
| | 3rd instar | 12 | 75.20 | 0.269 8* |
| | | 24 | 36.88 | 0.3644* |
| | 4th instar | 12 | 118.08 | 0.320 7 |
| | | 24 | 75.48 | 0.335 9* |

# Values were based on four concentrations with 30 insects each. # Control groups showed no mortality. *Significant at *P*<0.05 level.

## CONCLUSION

This study indicates that the ethanolic extract, petroleum ether and chloroform extract of buds and calyx of *Tagetes erecta* has larvicidal properties and its use as a larvicide against the dengue vector. *Aedes aegypti* mosquito should be explored. The percentage mortality increased with increasing concentrations of the buds and calyx of *Tagetes erecta* ethanolic extract, petroleum ether and chloroform fraction and also increased in relation to the time of exposure. Identification, isolation and mass synthesis of bioactive compounds of plant origin against mosquito menace are imperative for the management of mosquito-borne diseases. In addition, novel drug delivery systems of plant based active substances are need of time. Identifying plant based insecticides that are efficient as well as suitable and adaptive to local ecological conditions, biodegradable and have the wide spread larvicidal property will work as a new weapon in the arsenal of insecticides and in the future may act as a suitable alternative product to fight against mosquito-borne diseases.

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