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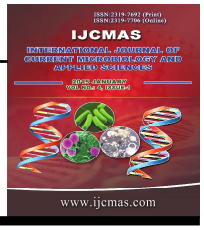

Original Research Article

Analysis of phytochemical components and larvicidal activity of *Thevetia peruviana* (Pers) Merr, against the chickungunya vector *Aedes aegypti* (L)

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K e y w o r d s

Aedes aegypti, *Thevetia peruviana*, Dengue, Phytochemicals and GC-MS.

A B S T R A C T

In the present study larvicidal efficacy of methanol leaf extract of *Thevetia peruviana* was tested against the aquatic stages of *Aedes aegypti.* The mortality, LC_{50} and LC_{90} values were noticed against I, II, III, IV instar larvae and pupae of *A. aegypti* after 24 hours. The secondary metabolites of *T. peruviana* which are responsible for larvicidal and pupicidal bioassay were qualitatively and quantitatively estimated. In addition, eight phytochemical compounds were noticed by gas-chromatography mass spectrometry.

Introduction

Thevetia peruviana an evergreen shrub, belonging to Apocynanceae family, is a very poisonous shrub in nature and the kernels being the most toxic. This plant is native of central and South America, but now frequently grown throughout the tropical. The shrub or small tree that bears yellow or orange-yellow, trumpet like flowers and its fruit is deep red/black in color enhancing a large seed that bears some resemblance to a Chinese "lucky nut". Leaves are covered in waxy coating to reduce water loss. The active principles in yellow oleander are cardiac glycosides. The physical properties of the fruit and kernel are unique and different from other tree borne oilseeds *i.e.* the storage, transport handling *etc*. Activities related to the fruits and kernels will require modifications in the processes and structures prevailing for other tree born oilseeds (Balusamy and Manrappan, 2007).

All parts of the plant are having medicinal value. *T. peruviana* contains a milky sap containing a compound called 'thevetin' that is used as a heart stimulant and other components are cardenolides called Thevetin A and B (cereberoside), peruvoside, nerrifolin, thevetoxin and ruvoside. These cardenolides are not destroyed by drying or heating and they are similar to digoxin from *Digitalis purpurea*. The

extracts and preparation from the plant which are hopefully safe, exhibited various additional biological effects such as antioxidant, immunomodulatory, anticancerogenic, antimicrobial, antiparasitic and insect antifeedant or repellent activities. Hence in the present study an effort has been made to assess the larvicidal activity of *T. peruviana* against the aquatic stages of medically important vector *A. aegypti.*

Materials and Methods

Eggs of *A. aegypti* were collected from the disease free standardized colony at National Communicable Diseases (NICD), Tamil Nadu and it was maintained in sterilized containers with unchlorinated tap water. The colony from this culture was used for further studies. The leaves of *T. peruviana* were collected from the field and chopped into small pieces with the help of a knife and dried under shade at room temperature (27 ± 2^0C) for about 20 days. The completely dried leaves were powdered with an electrical blender and sieved to get fine powder. The powder was stored in airtight containers for further analysis. The plant powder was extracted with methanol by using Soxhlet apparatus for 8 hours. The extracts were concentrated using a vacuum evaporator at 45^0C under low pressure. After complete evaporation of the solvent, the concentrated extract was collected and stored in glass vials at 4^0C in refrigerator for further experiments.

One gram of concentrated extract was dissolved in 100 ml of the methanol, kept as a stock solution. This stock solution was used to prepare the desired concentrations of the extract for exposure of the mosquito larvae. The larvicidal bioassay was done using standard WHO Protocols (WHO, 2005). Mortality in control was negligible in calculation. The percentage of larval and pupal mortality was corrected by Abbot's formula (Abbott, 1925). LC_{50}, LC_{90} were calculated from toxicity data by using probit analysis (Finney, 1971). The extracts were subjected to preliminary phytochemicals tests to determine the groups of secondary metabolites present in the plant materials as follows alkaloids, carbohydrate, steroidal glycosides, saponis, tannin, phenol, chlorogenic acid, flavonoids, coumarim, anthocyanin and terpenoid (Harborne, 1998). Based on the preliminary phyotochemical analysis alkaloid (Harborne, 1973), phenol (Li *et al*., 2008), flavonoid (Ozsoi *et al.,* 2008), steroid (Evans, 1996) and terpenoid were quantitatively estimated.

Result and Discussion

Bioassay tests were conducted to find out the effect of *T. peruviana* plant extract on the larval forms (I, II, III and IV) and pupae of *A. aegypti* were treated with different concentrations (500-700ppm) for 24 hours. The LC_{50} and LC_{90} values of methanol extract of *T. peruviana* were 473.18, 636.42 ppm for I-instar larvae; 536.48, 612.54 ppm for II-instar larvae; 498.29, 640.55 ppm for III-instar larvae; 572.74, 683.30 ppm for IV-instar larvae and 491.53, 638.29 ppm for pupae respectively (Table 1; Fig.1).

In the present study IV instar larvae of *A. aegypti* showed least susceptibility than pupae and larval stages against the methanol leaf extract of *T. peruviana*. This may clearly support the insect age plays an important role in influencing the susceptibility to pesticides and plant extracts (Umavathi and Manimegalai, 2010).

Table.1 Larvicidal effect of methanol leaf extract of *T. peruviana* against different larval instar and pupae of *A. aegypti* treated with 24 hours

Developmental stages	% of mortality						LC_{50} (ppm) (LCL-UCL)	LC_{50} (ppm) (LCL-UCL)	Regression equation	Chi-Square (χ^2)
	500 ppm	550 ppm	600 ppm	650 ppm	700 ppm	CD ($p<0.05$)				
I – Instar	55^{d}	70^{c}	80^{b}	100^{a}	100^{a}	1.564	473.18 (352.75 – 624.88)	636.42 (524.78 – 759.16)	y = 0.24 - 63 x	0.526
II - Instar	40^{d}	45^{c}	70^{b}	100^{a}	100^{a}	1.629	536.48 (405.34 – 753.42)	612.54 (518.37 – 706.25)	y = 0.35 - 139 x	0.543
III – Instar	35^{e}	40^{d}	70^{c}	90^{b}	100^{a}	2.105	498.29 (374.12 – 623.94)	640.55 (538.37 – 752.62)	y = 0.3 - 100 x	0.613
IV – Instar	25^{e}	35^{d}	65^{c}	75^{b}	95^{a}	2.443	572.74 (448.19 – 702.50)	683.30 (594.31 – 774.65)	y = 0.36 - 157 x	0.442
Pupae	45^{d}	70^{c}	85^{b}	100^{a}	100^{a}	1.729	491.53 (370.79 – 612.42)	638.29 (549.72 – 727.51)	y = 0.28 - 88 x	0.460

Values are expressed by mean ± SD of five samples in each column

$^{a-e}$ Mean values with in a row, no common superscript differ significantly at 5% by DMRT

Table.2 Preliminary phytochemicals analysis of methanol leaf extract in *T. peruviana*

S. No	Phytochemical Constituents	Name of the Test	Plant extract
1	**Alkaloid**	Mayer's test Dragendroff's test Wagner Test	+ + +
2	**Carbohydrate**	Molish Test Fehling Test Benedicts Test	- - -
3	**Steroidal Glycosides**	Libermann's test Salkowaski test	- +
4	**Saponin**	Foam Test	-
5	**Tannin**	Lead Acetate	-
6	**Phenol**	Phenol reagent	+
7	**Chlorogenic acid**	Ammonia test	+
8	**Flavonoids**	Ammonia test	+
9	**Coumarin**	Sodium chloride test	-
10	**Anthocyanin**	H_2So_4 test	-
11	**Terpenoid**	Borntrager's test	+

\+ Present of compounds; - Absent of compounds

Fig.1 Regression line for lethal concentration values of methanol leaf extract of *T. peruviana* against the aquatic stages of *A. aegypti* treated for 24 hours

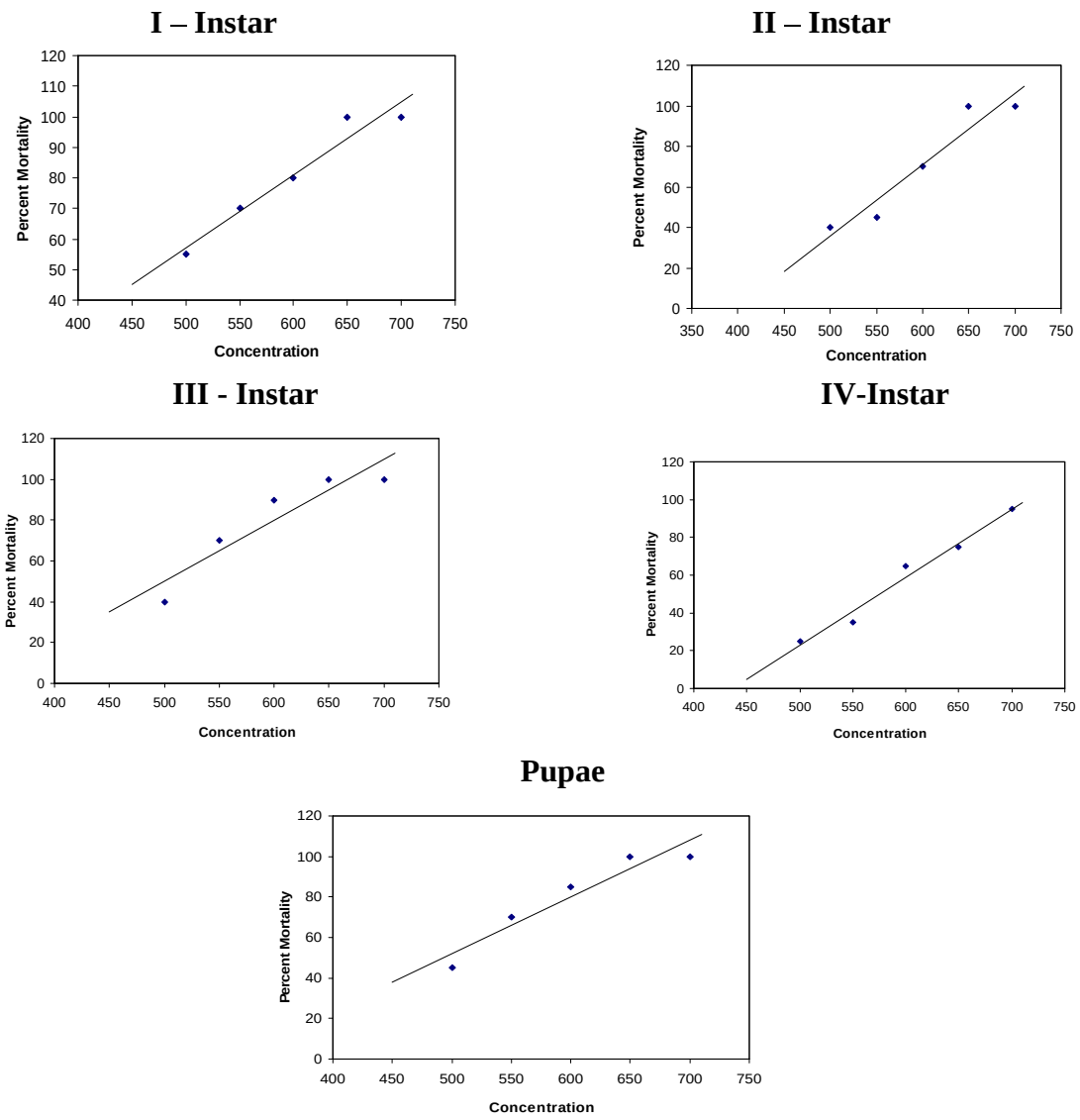

Table.3 Quantitative analysis of phytochemicals in methanol leaf extract of *T. peruviana*

S.NO	Name of the samples	Values (%)
1	Alkaloid	46.8
2	Phenol	38.4
3	Flavonoids	30.8
4	Steroid	20.0
5	Terpenoid	17.7

Fig.2 Gas chromatogram of methanol leaf extract of *T. peruviana*

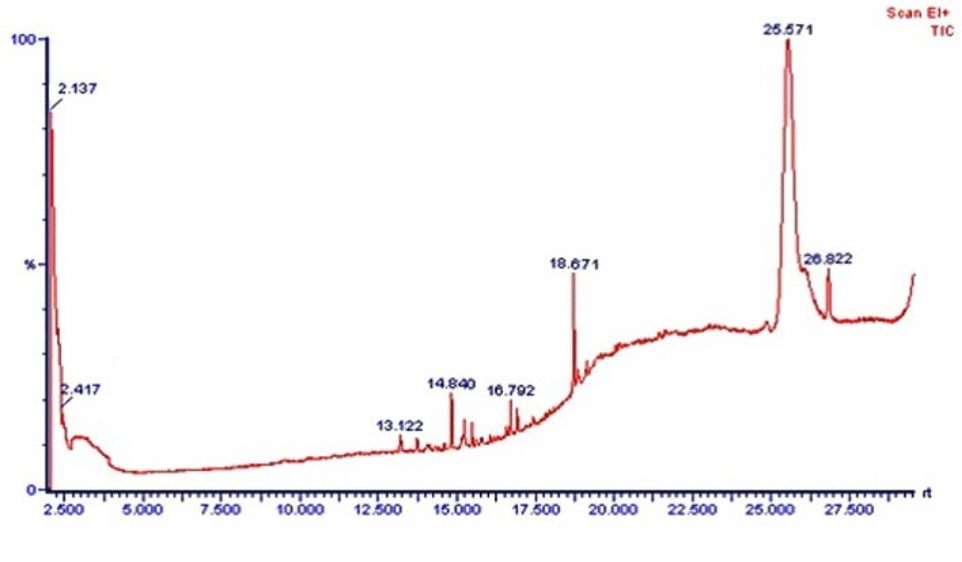

Table.4 GC-MS analysis of methanol leaf extract of *T. peruviana*

S. No.	Compound name	Molecular formula	Molecular weight
1	8-Hydroxy-6, 7-epoxydendrolasin	$C_{15}H_{22}O_3$	250
2	Citronellyl tiglate	$C_{15}H_{26}O_2$	238
3	Methyl 16-hydroxy-3-3-dimethylhepatadecanoate	$C_{20}H_{40}O_3$	328
4	1-xAllyloxy-1-ethynyl-5-methylcyclohexane	$C_{15}H_{24}O$*	220
5	2-(Methoxycarbonyl)-2-propargyl-1-cyclopentanol	$C_{10}H_{14}O_3$	182
6	1, 2-Benzenedicarboxylic acid, dicyclohexyl ester	$C_{20}H_{26}O_4$	330
7	1-(-R)-endo-Methylbornyl E-butenoate	$C_{15}H_{24}O_2$	236
8	1, 3, 4, 5-Tetramethylbicyclo [3.2.0] hex-3-ene-2-one	$C_{10}H_{14}O$*	150

The results revealed that the mortality rate was increased after the increase of concentration and the larvae also undergo malanization slowly. On the other hand, Al-Sharook *et al.* (1991) reported that the death of treated insects may be due to the inability of the molting bodies to swallow sufficient volume of air to split the old cuticle and expand the new one during ecdysis or to a metamorphosis inhibiting effect of the plant extract which is possibly based on the disturbance of the hormonal regulation.

The 100% mortality might be due to the chemical constituents present in the methanol leaf extract *T. peruviana* that

arrest the metabolic activity of the larvae, which caused the high percentage of mortality. Earlier authors reported that the methanol extract of *C. fistula* exhibited LC_{50} values of 17.97 and 20.57 mg/L, *Anopheles stephensi* and *Culex quinquefasciatus*, respectively (Govindarajan *et al.* 2014).

The methanol extract of *T. peruviana* was subjected to preliminary phytochemical analysis. The result showed the presence of alkaloid, glycosides, phenol, chlorogenic acid and terpenoid but **c**arbohydrate, steroidal, saponin, tannin, flavonoids, coumarin and anthocyanin were absent in methanol leaf extract of *T. peruviana* (Table 2). Based on the preliminary phytochemical analysis the quantified secondary metabolites are alkaloid, phenol, flavonoids, steroids and terpenoid. In quantitative studies 46.8% of alkaloid, 38.4% of phenol, 30.8% of flavonoids, 20.0% of steroid and 17.7% of terpenoid is present in methanol leaf extract of *T. peruviana* (Table 3).

The chemical components of methanol extract of *T. peruviana* were analyzed by Gas Chromatography Mass Spectrum (GC-MS). Chemical components are listed in the Table 4 and eight components were identified with different molecular weights (Fig.2). Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, which makes it easy to deal with them in this habitat. The use of conventional pesticides in the water sources, however, introduces many risks to people and environment. Natural pesticides, especially those derived from plants, are more promising in this aspect. Aromatic plants and their essential oils are very important sources of many compounds that are used in different aspects (Amer and Mehlhorn, 2006).

Secondary metabolites produced in plants for its protection against microorganisms and predator insects are natural candidates for the discovery of new products to combat *A. aegypti*. Several studies have focused on natural products for controlling *A. aegypti* mosquitoes as insecticides and larvicides, but with varied results (Sarita *et al.,* 2011; Rajan and Savarimuthu, 2012; Raveen *et al.,* 2012).

Compared to control groups, behavioral changes were noticed in *T. peruviana* treated group. This may due to the presence of neurotoxins in the plant extract. Crude extracts or isolated bioactive phytochemicals from the plant could be used in stagnant water bodies which are known to be the breeding grounds for mosquitoes. However, further studies on the identification of the active principals involved and their mode of action and field trials are usually needed to recommend *T. peruviana* as an anti-mosquito product used to combat and protect from mosquitoes in a control program.

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