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TOXICITY OF *TRIDAX PROCUMBENS* LEAF EXTRACT TO DENGUE VECTORS *AEDES AEGYPTI* L. AND *AE ALBOPICTUS* SKUSE

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ABSTRACT

Insecticides of plant origin are effective in mosquito control owing to its mode of action. In the present study, *Tridax procumbens* L. ethanolic leaf extract was found to be toxic to the larvae of *Aedes aegypti* L. and *Ae. albopictus* Skuse as these caused 100% mortality in *Ae. aegypti* and 97% mortality in *Ae. albopictus* after 72 hr, with LC_{50} values of 288.40, 120.33 and 77.62 mg/ l for *Ae. aegypti*, and 812.83, 338.84 and 128.83 mg/ l for *Ae. albopictus* after 24, 48 and 72 hr, respectively. The toxicity of *T. procumbens* ethanolic leaf extract on larvae of *Aedes* spp., could be attributed to the presence of phytochemical compounds, viz., betulin, betulinic acid, lupeol (triterpenoid), caryophyllene, isophytol, phytol (terpene), limonene (monoterpene), luteolin (flavonoid), stigmast-5-en-3-ol, 3 (Beta)-, stigmast-5-en-3-ol, oleate, and palmitic acid (sterol) revealed by the gas chromatography-mass spectrometry analysis.

Key words: *T. procumbens*, leaf, ethanol, Gas chromatography-mass spectrometry (GC-MS), phytochemical constituents, *Ae. aegypti*, *Ae. albopictus*, larvicidal, toxicity

Man suffers extensively due to the nuisance of insect, particularly mosquitoes in health point of view as they directly transmit diseases (WHO, 2017; Chala and Hamde, 2021). *Aedes* is a genus of mosquitoes, originally found in tropical zones of Southeast Asia including India. *Aedes aegypti* and *Ae. albopictus* are responsible for the transmission of dengue fever. According to WHO (2021), the diseases transmitted by *Aedes* spp., are serious in the field of public health. The principal method by which mosquito/ vector-borne diseases are controlled is through vector control, which has a long and distinguished history (Wilson et al., 2020). There is a need to return to vector control approaches, which utilize a range of insecticides. Synthetic chemicals are effective, nonetheless, cause adverse effects on the environment and human health (van den Berg et al., 2021). Due to their hazardous side effects, ecofriendly alternatives are required for safer mosquito management. One such alternative approach is to explore the floral biodiversity and use these as insecticides of botanical origin (Nathan, 2020). Such a search for natural mosquitocides is ongoing, as the phytochemicals from plant origin have multiple modes of action (Smith et al., 2021). The development of botanical insecticides have become more rigorous in recent years with calls for more standardization, especially against mosquito larvae (Shaalan et al., 2005; Sakthivadivel and Daniel, 2008; Samuel et al., 2012a, b; Arivoli et al., 2012a, b; Ghosh et al., 2012; Kishore et al., 2014; Samuel and William, 2014; Raveen et al., 2017; Pavela et al., 2019; Nathan, 2020). In addition to the direct use of phytoextracts, biosynthesized phytonanopesticides are also gaining momentum as biocontrol agents against mosquitoes (Samuel et al., 2016). *Tridax procumbens* extracts have proved to be effective against mosquito larvae, however few reports present the larvicidal activity of its ethanolic extract (Macedo et al., 1997; Elumalai et al., 2013). Therefore, *T. procumbens* ethanolic leaf extract's phytochemical profile (GC-MS) analysis, and its toxicity against *Ae. aegypti* and *Ae. albopictus* larvae have been assessed in this study.

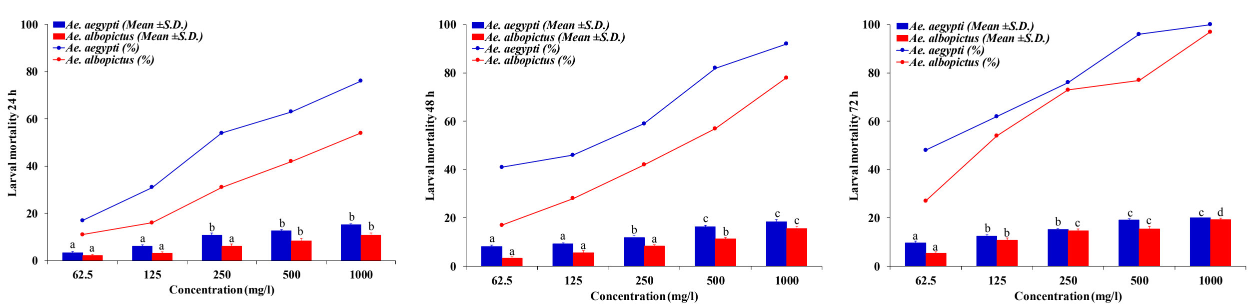
MATERIALS AND METHODS

Mature, fresh and healthy leaves of *T. procumbens* collected from Nagercoil, Kanyakumari, Tamil Nadu, India were brought to the laboratory, and taxonomically identified at the Department of Botany, Scott Christian College, Nagercoil, Kanyakumari, Tamil Nadu, India. The collected leaves were washed with dechlorinated water, and shade dried at room temperature. Thereafter, the dried leaves were coarsely powdered by an electric blender, and sieved by a kitchen sieve. Finely powdered leaf (1 kg) was extracted with ethanol (3 l) in a Soxhlet apparatus. The crude ethanolic leaf extract thus obtained was stored in air tight amber-coloured bottles at 4°C for bioassay. Clarus 680 GC was used for GC-MS analysis of *T. procumbens* ethanolic leaf extract to obtain its phytochemical profile in the Sophisticated Instrumentation Facility, Chemistry Division, School of Advanced Science, VIT University, Vellore, Tamil Nadu, India. *Aedes* immatures collected from Nagercoil, Kanyakumari, Tamil Nadu, India with an aid of a dipper were transported in plastic containers to laboratory, and thereafter moved to enamel larval salvers till adult emergence. Adults were identified and species confirmed prior to rearing (Tyagi et al., 2015). Subsequently, their cyclical generations were provided a blood meal, and was maintained in two feet mosquito cages (27 ±2°C, 70-80% RH) inside an insectary. Ovitraps inside the mosquito cages collected the oviposited eggs which were shifted to the larval rearing room in enamel larval salvers, and the larvae on hatching were provided larval food (yeast and dog biscuits of ratio 1:3). The larvae on turning into pupae were moved to another mosquito cage in enamel bowls for adult emergence.

World Health Organization (WHO) protocol was adopted for the study with minor modifications (WHO, 2005). Serial dilution of 1.0% stock solution of the crude phytoextract yielded requisite test concentrations (62.5, 125, 250, 500, and 1000 mg/ l) and amount of test solution. Early third instar larvae obtained from laboratory colonized F_1 generation was tested. The early third instars numbering 20 were added into glass beakers (250 ml) holding distilled water and test concentration for each replicate apiece trial. Distilled water (250 ml), and Tween 80 (1.0 ml) dissolved in distilled water (249 ml) maintained separately and run simultaneously served as positive and negative control, respectively. Larval mortality was confirmed when the moribund larvae showed no signs of movement when prodded by a needle on their respiratory siphon, and were scored dead. Mortality was observed 24, 48 and 72 hr after treatment. A total of five replicates, a negative and positive control were run concurrently for every trial, and overall five trials were run. Larval mortality in % was calculated, and when control mortality ranged from 5-20%, it was corrected by Abbott's (1925) formula. All mortality data were subjected to probit analysis, chi-square and regression analysis. One-way analysis of variance with Tukey's honestly significant tests was done to differentiate mean mortality. The differences were considered as significant at $p \leq 0.05$ level. Statistical analyses were carried in IBM SPSS Statistics v22 (SPSS, 2010).

RESULTS AND DISCUSSION

The phytochemical profile of *T. procumbens* ethanol leaf extract by GC-MS analysis revealed the presence of flavonoids, phenols, saponins, steroids, sterols, tannins, terpenes and terpenoids, and its phytochemical compounds were betulin, betulinic acid, caryophyllene, hydroquinone, isophytol, limonene, linoleic acid, lupeol, luteolin, myristic acid, 4-octanol, oleic acid, palmitic acid, pentadecanoic acid, phytol, salicylic acid, stearic acid, stigmast-5-en-3-ol, 3 (Beta)-, stigmast-5-en-3-ol oleate, squalene, tridecylic acid, and undecylic acid. The mortality of *Ae. aegypti* and *Ae. albopictus* larva exposed to various concentrations of *T. procumbens* ethanolic leaf extract after 24, 48 and 72 hr are presented in Fig. 1. No larval mortality was observed in both controls. Complete mortality was observed in *Ae. aegypti* larvae followed by 97% in *Ae. albopictus* at the highest concentration after 72 hr of exposure. *T. procumbens* extracts gave LC_{50} values of 288.40, 120.33 and 77.62 mg/ l for *Ae. aegypti* and 812.83, 338.84 and 128.83 mg/ l for *Ae. albopictus* after 24, 48 and 72 hr, respectively. One way ANOVA, comparing treated and control group ($p<0.05$) showed that *T. procumbens* concentrations significantly influenced the mortality of *Aedes* larvae (Table 1). Overall results



Different superscript alphabets indicate statistical significant difference in larval mortality between concentrations at $p<0.05$ level, one way ANOVA followed by Tukey's test

Fig. 1. Larval mortality of *Aedes* spp. on exposure to *T. procumbens* ethanolic leaf extract

Table 1. Probit analysis and other associated statistical inferences for the present study

Hours	LC_{50} (mg/l)	LC_{90} (mg/l)	Chi-square	Regression equation	R^2	F	P
				Ae. aegypti			
24	288.40	2454.70	0.418	Y=0.011X+5.300	0.7943	4.912	<0.057
48	120.23	954.99	1.234	Y=0.011X+8.525	0.8905	4.830	<0.059
72	77.62	380.19	1.596	Y=0.010X+11.317	0.7775	4.770	<0.600
				Ae. albopictus			
24	812.83	4069.78	0.135	Y=0.008X+2.725	0.895	5.003	<0.055
48	338.84	2754.23	0.214	Y=0.012X+4.141	0.939	4.932	<0.057
72	128.83	616.60	1.642	Y=0.012X+8.441	0.757	4.822	<0.059

indicated that the *T. procumbens* ethanolic leaf extracts are more toxic on *Ae. aegypti* than *Ae. albopictus*.

The results of the present study were comparable with the earlier reports of *T. procumbens* ethanolic extracts against mosquito larvae. Macedo et al. (1997) screened ethanolic aerial extracts of 83 plants belonging to Asteraceae family for larvicidal activity against *Aedes fluviatilis* Lutz of which 27 caused significant lethality. Elumalai et al. (2013) reported its aqueous, chloroform, ethanol, petroleum ether and methanolic leaf extracts to possess larvicidal activity against *Ae. aegypti* (LC_{50} 83.40, 108.22, 55.67, 94.13 and 56.02 ppm), *An. stephensi* (LC_{50} 92.79, 104.73, 77.70, 117.09 and 66.66 ppm) and *Cx. quinquefasciatus* (LC_{50} 80.58, 97.93, 57.46, 111.48 and 60.31 ppm). The susceptibility of larvae to botanical insecticides depends in general on the solvent extract, and the mosquito species tested. In order to get a potent extract, prior to selection of solvents, a thorough knowledge on the phytochemical profile of the plant/plant part used should be drawn, as there exists a relationship between the extract effectiveness and solvent polarity. The choice of solvent is influenced by what is intended with the extract, as it targets the compounds to be extracted (Ghosh et al., 2012). In the present study, ethanol, an intermediary solvent with a polarity index of 5.2 extracted bioactive phytocompounds responsible for toxicity of mosquito larvae. Ethanol has the property to extract alkaloids, coumarins, flavonoids, phenols, quinines, saponins, sterols, tannins, terpenes and terpenoids which are toxic to the immature mosquitoes (Shaalan et al., 2005).

Samuel et al. (2018) provided an exhaustive review on the list of ethanolic plant extracts reported for mosquito larvicidal property, and in the present study *T. procumbens* ethanolic leaf extract against *Ae. aegypti* and *Ae. albopictus* larvae (first time) has been reported for its toxicity in this study. An examination into the larvicidal mode of action by these phytochemicals on species of *Aedes* mosquito larvae include, direct attack and damage on the nervous system, affect the midgut epithelium primarily, and affect the gastric caeca and the Malpighian tubules secondarily (Rey et al., 1999), act as mitochondrial poison (Mann and Kaufman, 2012) and work by interacting with cuticle membrane of the larvae ultimately disarranging the membrane which is the most probable reason for larval death (Hostettmann and Marston, 1995). In the present study, *T. procumbens* extracts caused mortality to *Ae. aegypti* and *Ae. albopictus* larvae which can be attributed to the presence of its phytochemical compounds, viz., betulin, betulinic acid, lupeol (triterpenoids), caryophyllene, isophytol, phytol (terpenes), limonene (monoterpene), luteolin (flavonoid), stigmast-5-en-3-ol, 3 (beta)-, stigmast-5-en-3-ol, oleate, and palmitic acid (sterols). Thus, *T. procumbens* extracts possess lethal effects against larvae of *Aedes* spp., and this study corroborates the findings of da Silva et al. (2016) who reported on the action of triterpenoids on *Ae. aegypti* larvae. Further, Samuel et al. (2020) reported that the flavonoids, tannins, limonoids (terpenoids) of *Citrus limon* (L.) Osbeck leaf extracts arrested the metabolic activities of *Ae. aegypti* larvae, inhibited its skin changes, disrupted the body's metabolism which resulted in lack of energy for life activities, and caused the *Ae. aegypti* larvae to spasm and eventually in its death. The same was observed in the present study too. Selection of mosquito species for testing is also of fundamental importance since great variations exist in responses between the genera and species, and in the present study Aedines were selected as they are the most commonly colonized mosquitoes which are less susceptible to insecticides.

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