EFFICACY OF DIFFERENT PARTS OF *Tridax procumbens* AS A POTENTIAL BIOLARVICIDE AGAINST *Aedes aegypti* AND *Aedes albopictus*

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ABSTRACT

The epidemic of dengue and Zika outbreak transmitted by *Aedes* mosquitoes has been a global issue and plenty of preventive measurements are being used. Due to environmental awareness, people are opting on bioinsecticide. The objective of this research is to study on the effectiveness of different parts of *Tridax procumbens* plant on *Aedes aegypti* and *Aedes albopictus* larvae. *Tridax procumbens* are endemic plants in Malaysia. Using WHO standard larval bioassay, third instar larvae of *Ae. aegypti* and *Ae. albopictus* were exposed to different concentrations, ranging between 250ppm-2500ppm for three different parts of *T. procumbens* plant extracts obtained from Soxhlet extraction. Larval mortality was observed after 24-hour exposure. The highest toxicity for *Ae. aegypti* was recorded by *T. procumbens* stem extract with the LC_{50} of 799.78ppm. The pairwise comparison showed, the highest mean mortality value for stem did not show any significant difference with leaves; however, it did show a significant difference with flowers when tested on *Ae. aegypti* (P<0.05). Meanwhile for *Ae. albopictus*, the highest toxicity recorded with the LC_{50} of 583.63ppm was by the *T. procumbens* flower extract. The mean mortality value for flower showed significant differences from other plant parts when tested on *Ae. albopictus* (P<0.05). This indicates that *T. procumbens* is effective in killing both *Ae. aegypti* and *Ae. albopictus*.

Keywords: *Aedes,* biolarvicide, mosquito, plant extract, *Tridax procumbens*

ABSTRAK

Wabak denggi dan Zika yang ditular oleh nyamuk *Aedes* telah menjadi isu global dan pelbagai langkah pencegahan sedang digunakan. Disebabkan kesedaran akan alam sekitar, ramai orang memilih untuk menggunakan bioinsektisid. Objektif kajian ini adalah untuk mengkaji keberkesanan tumbuhan *Tridax procumbens* terhadap larva *Aedes aegypti* and *Aedes albopictus*. *Tridax procumbens* adalah tumbuhan di Malaysia. Menggunakan bioasai larva WHO, larva instar ketiga *Ae. aegypti* dan *Ae. albopictus* telah didedahkan kepada beberapa jarak kepekatan yang berbeza sekitar 250ppm-2500ppm untuk ekstrak tiga bahagian *T. procumbens* yang diekstrak daripada pengekstrakan Soxhlet. Kematian larva telah dilihat 24 jam selepas pendedahan. Ketoksikan tertinggi bagi *Ae. aegypti* telah direkodkan oleh ekstrak batang *T. procumbens* dengan LC_{50} sebanyak 799.78ppm. Perbandingan berpasangan telah menunjukkan nilai kematian tertinggi bagi batang tidak menunjukkan sebarang perbezaan signifikan dengan daun, tetapi ia menunjukkan perbezaan signifikan dengan bunga apabila

diuji ke atas *Ae. aegypti* ($P < 0.05$). Sebaliknya untuk *Ae. albopictus*, ketoksikan tertinggi dicatat dengan LC_{50} sebanyak 583.63ppm oleh ekstrak bunga *T. procumbens*. Nilai kematian tertinggi bagi bunga telah menunjukkan perbezaan signifikan berbanding bahagian lain apabila diuji ke atas *Ae. albopictus* ($P < 0.05$). Ini menunjukkan bahawa *T. procumbens* berkesan dalam membunuh *Ae. aegypti* dan *Ae. albopictus*.

Kata kunci: *Aedes*, bio-larvisid, ekstrak tumbuhan, nyamuk, *Tridax procumbens*

INTRODUCTION

Vector-borne diseases from mosquitoes such as dengue fever, yellow fever and malaria has caused a significant number of global infectious disease burden with nearly 3.5 billion people around the globe are at risk of being infected by at least one type of the vector-borne pathogen (Hales et al. 2002; WHO 2004). The epidemic outbreak of dengue and Zika has brought concerns to people worldwide. In Malaysia, there have been 41,574 dengue cases with 97 deaths reported up till May 2017 (WHO 2017). Due to the alarming rate of the number of dengue cases, preventive measures are being researched to halt the outbreak which include source reduction, environmental management, chemical control, personal protection, physical barrier and biological control (Sharma & Singh 2008).

Chemical control is one of the major types of control being used in controlling the increasing number of *Aedes* mosquitoes which includes chemical larviciding, mosquito coil, aerosol and thermal fogging (WHO 2003). The usage of synthetic chemicals imposes a threat to human health and the environment as such chemicals tend to persist in the environment for a long time in addition to causing resistance among pest insects. The persistence causes poisoning to both the environment and human being. Such an issue has caused researchers to look for alternatives to replace these synthetic chemicals.

Recently, extractions from plants derivatives have proven to be possible alternatives bio-larvicides (Rahuman et al. 2008). It has been reported on a previous study that, plant-based insecticide like *Ipomoea cairica* leaf extract is effective in controlling *Aedes* larvae (Zuharah et al. 2018). In contrast to the conventional synthetic chemicals which consist of a single active ingredient, these plant-based bio-insecticides has a mixture of chemical compounds that react on the physiological and behavioural process of the insects (Anupam et al. 2011). The usage of such plant based bio-insecticide is economically healthy especially when the plant sources are abundant like common weeds, shrubs and trees with huge distribution (Taha et al. 2011). Among these plants is *Tridax procumbens* where the plant has been reported to have effective repellent activity against mosquitoes (Kamaraj et al. 2011), with larvicidal properties towards *Ae. aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Devan et al. 2013). In the study by Devan et al. (2013), the methanolic extract of the whole plant of *T. procumbens* was effective in killing *Ae. aegypti* and *Cx. quinquefasciatus* larvae at 53 ppm. Whereas, the study by Kamaraj et al. (2011) revealed that the leaf acetone extract of *T. procumbens* is toxic to *Anopheles subpictus* at 51.57 mg/ml and the leaf ethyl acetate extract on *Culex tritaeniorhynchus* at 69.16 mg/ml. However, both studies did not mention about the properties of *T. procumbens* that are responsible for larvicidal effects. Thus, it indicated that the compound of the *T. procumbens* plant responsible for having the larvicidal properties is still unknown. It's also known to be used to treat malaria and high blood pressure (Jude et al. 2009). The objective of this study is to evaluate and compare the effectiveness of methanolic extracts of different parts of *T. procumbens* plant, namely flower, stem and leaves *Ae. aegypti* and *Ae. albopictus* larvae using larval bioassay technique.

MATERIALS AND METHODS

Collection and Extraction of *Tridax procumbens*

Tridax procumbens plants were collected around the main campus of Universiti Sains Malaysia (5° 21' 20.52"N, 100° 18' 4.32"E). The collected plants were the separated into three different parts, flowers, stems and leaves. The separated parts were dried under shade for 14 days and blended into fine powder using commercial electrical stainless steel blender (Panasonic: MX-899TM). The powder was placed into the Soxhlet apparatus with methanol as solvent and the extraction was conducted about 3 to 4 cycles to ensure maximum yield. A total of 2 litres of methanol and 40g of the sample were used in each extraction. Methanol was also chosen as solvent as it was reported to be effective in extracting all polar and non-polar compounds in a plant during an extraction. Other than that, methanol extract was found to be more effective in the study by Devan et al. (2013) when compared with chloroform, ethanol and petroleum ether. Once the extraction completed, the extract was placed in the rotary evaporator for about 30 minutes at 66^{o}C at a speed of 100 rpm to allow all the methanolic solvent to evaporate leaving the crude extract behind. A stock solution of 10000ppm was made by diluting 1g of extracts into 10ml of methanol and 90ml of distilled water and placed in the refrigerator until further use. The process was done separately for each plant part.

Mosquitoes

Susceptible laboratory strains of *Ae. aegypti* and *Ae. albopictus* were used in this experiment which were provided by the Vector Control Unit, Universiti Sains Malaysia. The strain was cultured for more than 600 generations since 1980s. The eggs were immersed in separate enamel trays containing seasoned water. Seasoned water is tap water that is left in a container for several days to allow the chlorine in the water to evaporate. The eggs hatched within an hour after soaking in seasoned water. The hatched larvae were cultured in the laboratory at a temperature of 26±2°C and 70-80% of relative humidity. The larvae were fed 10mg of larval food daily. Food consisted of dog biscuit, beef liver, yeast and milk at a ratio of 2:1:1:1 by weight. The larvae were reared till they reached late L3 instars or early L4 instars. This larval stage was chosen for the experiment based on WHO standard larval bioassay (WHO 2005).

WHO Larval Bioassay Test Procedure

A preliminary test was conducted on both *Ae. aegypti* and *Ae. albopictus* larvae using different concentrations of the extract to obtain the minimum concentration to determine the 0% mortality and the maximum concentration to determine the 100% mortality of the tested larvae population for both species prior to establishing an actual range of concentrations for the experiment. The range of concentration required for the experiment was then determined based on the known minimum and maximum concentrations. A total of ten concentrations were used for this study for all the parts of *T. procumbens*; 250, 500, 750, 1000, 1250, 1500, 1750, 2000, 2250 and 2500ppm. A total of 20 larvae were placed in a 250ml paper cup containing 200ml at different concentration of extracts as mentioned above. Each paper cups were filled with extracts of the determined concentrations earlier, and each concentration was replicated three times for both larvae species and for respective parts of *T. procumbens*. Three replicates of controls were prepared by placing 20 larvae in a 250ml paper cup containing 1ml of methanol and 199ml of distilled water. The paper cups were then left for 24 hours at the standard laboratory condition; temperature: 28±3oC, relative humidity (RH) of 70±10%. Mortality counts for each paper cups were taken 24 hours' post treatment. Mortality count is taken by sieving the larvae and checking for any movement. Moribund larvae are counted and added to dead larvae for calculating percentage mortality. Dead larvae are those that are unable to move

when they are probed with a needle in the siphon or the cervical region meanwhile moribund larvae are those incapable of rising to the surface or not diving when the water is disturbed.

Data Analysis

All data were analysed using probit analysis in SPSS version 20.0 to obtain the lethal concentration 50 (LC_{50}) and lethal concentration 95 (LC_{95}). Data were log-transformed prior to statistical analysis to fulfil the assumption of probit analysis. LC_{50} was defined as the concentration required to kill 50% of the larvae, whereas LC_{95} is the concentration required to kill 95% of the larvae. The mortality values were analysed using two-way ANOVA to check for significant effects among the parts of *T. procumbens* extracts and concentration used.

RESULTS

The crude extracts of all the parts of *T. procumbens* show larvicidal properties on both *Ae. aegypti* and *Ae. albopictus*. The extract from the stem of *Tridax procumbens* worked best on *Ae. aegypti* as the LC_{50} and LC_{95} of the extract is 799.78ppm and 1506.66ppm respectively which is lower than the other flower and leaves extracts (Table 1). This can also be seen in Fig. 1 where the mortality line of the stem extract is higher compared to flower and leaves extracts. Post hoc Tukey test showed that there was no significant difference when compared between the stem extract and leaves extracts ($P>0.05$), however the stem extract was significantly higher in larval mortality when compared with the flower extract for *Ae. aegypti* larvae ($F=23.78$, $df=2$, $P=0.000$). Meanwhile for *Ae. albopictus* the flower extract of *Tridax procumbens* seems to have worked best with the LC_{50} and LC_{95} of 583.63ppm and 2329.41ppm and this can also be seen in Fig. 2 where the line of the flower is higher when compared with the remaining two parts. The flower extract was significantly higher in larval mortality between all the extracts used in the test ($F=55.02$, $df=2$, $P=0.000$). Multivariate Analysis of Variance (MANOVA) showed that there is a significant difference between species, parts of plant and concentrations indicating that there is an interaction effect between all the variables ($F=6.30$, $df=18$, $P<0.05$; Table 2). No mortality was recorded on the control treatments.

Table 1 Mean LC_{50} and LC_{95} (in ppm) of larval efficacy on *Aedes aegypti* and *Aedes albopictus* for three parts of *Tridax procumbens* plant after 24 hours of exposure (95% confidence limit).

Species	Parts used	LC_{50}	LC_{95}	Regression equation
Ae. aegypti	Leaves	780.04 (593.64-951.01)	2669.16 (2018.53-4348.04)	Y= -8.90+0.71X
	Stem	799.78 (664.30-916.82)	1506.66 (1283.16-1959.52)	Y= -17.55+1.55X
	Flower	966.98 (810.76-1119.17)	2773.28 (2217.89-3961.89)	Y= -10.73+0.81X
Ae. albopictus	Leaves	1324.39 (1160.16-1496.64)	3083.83 (2511.27-4353.22)	Y= -14.97+1.12X
	Stem	1089.08 (1006.04-1170.34)	2834.98 (2505.89-3327.22)	Y= -11.23+0.90X
	Flower	583.63 (434.42-717.29)	2329.41 (1793.25-3553.37)	Y= -7.57+0.67X

Table 2 Analysis of variance on larval mortality species, plant parts used and concentration

Source of variation	df	MS	*F*-value	*P*-value
Species	1	88.20	30.13	**0.000***
Parts of plant	2	51.04	17.73	**0.000***
Concentration	9	750.99	256.51	**0.000***
Species*Parts of plant	2	207.62	70.91	**0.000***
Species*Concentration	9	16.87	5.76	**0.000***
Parts of plant*Concentration	18	10.35	3.53	**0.000***
Species*Parts of plant*Concentration	18	18.43	6.30	**0.000***

*Significant values are given in bold.

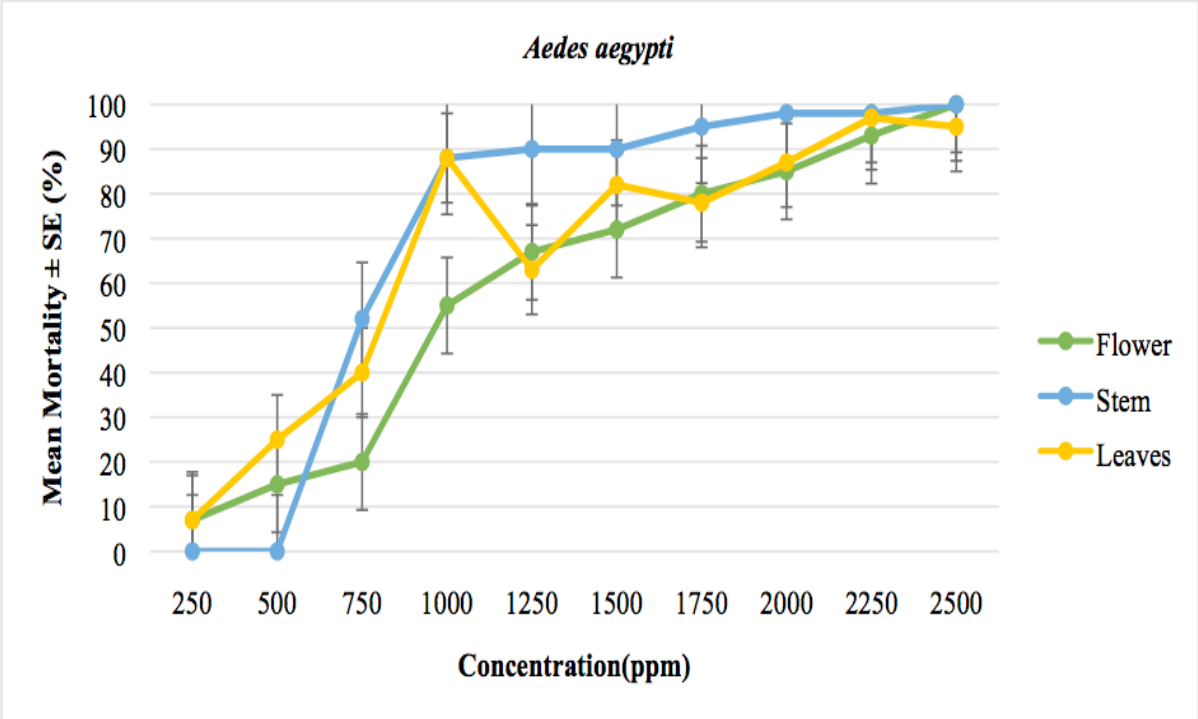

Figure 1 Dose response relationship for selected part of *Tridax procumbens* extracts, applied for 24 hours on *Ae. aegypti*

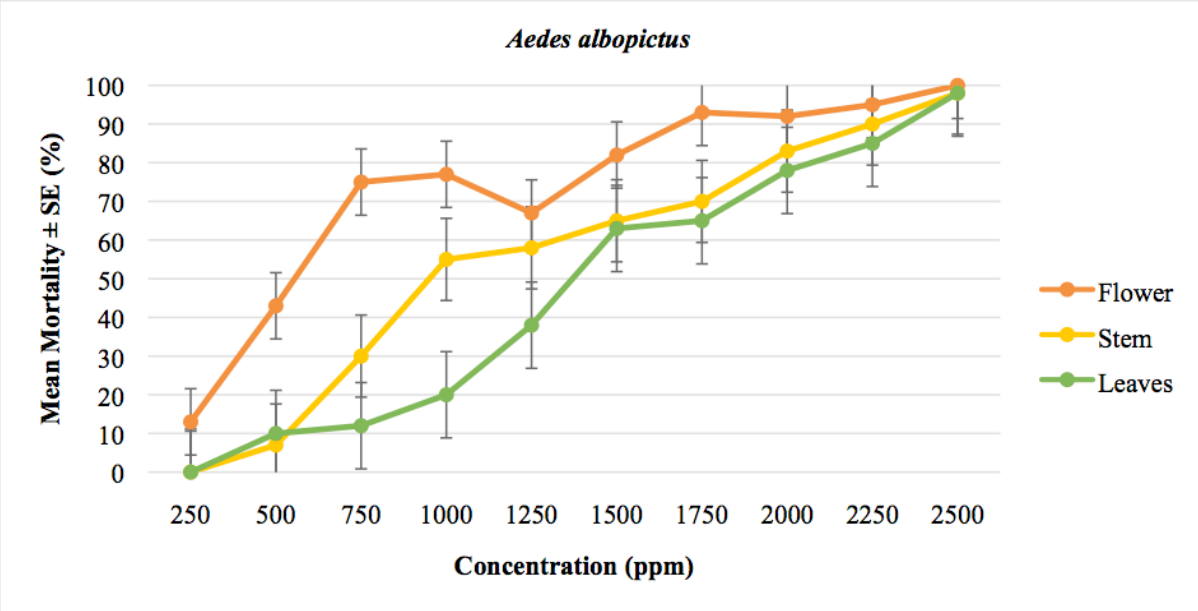

Figure 2 Dose response relationship for selected part of *Tridax procumbens* extracts, applied for 24 hours on *Ae. albopictus*

DISCUSSION

Our results revealed that the extracts from the stems of *T. procumbens* are effective in controlling *Ae. aegypti* while extracts from the whole plant of *T. procumbens* are effective in controlling *Ae. albopictus* at a higher dosage. Different parts of the *Tridax procumbens* plant affected differently of *Ae. aegypti* and *Ae. albopictus* as it was researched that both species of *Aedes* larvae are known to react differently with similar chemicals. The susceptibility difference in the different parts of *T. procumbens* against the two species is because it was reported *Ae. albopictus* to be more tolerant to insecticides than *Ae. aegypti* (Gomez et al. 2011). This study has been reported lower lethal concentrations of *T. procumbens* of *Ae. albopictus* and *Ae. aegypti* than those reported in the study by Devan et al. (2013) in India. This variation in efficacy of *T. procumbens* is known to be common as the toxicity and the concentration of active compound in *T. procumbens* varies by the geographical distribution of the plant, extraction method, types of solvent used and the plant parts used in extraction (Sukumar et al. 1991).

In contrary to most researches (Edwin et al. 2013; Wilson et al. 2014), we found the extracts from the stem of *Tridax procumbens* showed better effectiveness than leaves extract when tested on *Ae. aegypti*. However, it is possible that extracts from the stems to be more effective than the leaf extracts when tested on *Ae. aegypti* (Kumar et al. 2012). When tested on *Ae. albopictus* it was found that extracts from the flower worked best on the larvae. It was proven earlier that extracts from flowers of plants have effective larvicidal properties on *Aedes* larvae (Kamaraj et al. 2011; Eugeni et al. 2014).

The fluctuations on the larval mortality for leaves when tested against *Ae. aegypti* and for aerial and flower parts when tested against *Ae. albopictus* is due to accumulation of the bioactive compound on certain part of the plant (Shaalan et al. 2005). In which this situation also happened in this study. The bioactive compounds are essential in the mortality of the *Aedes* larvae. The bioactive compound accumulation in different part of the plant causing in the

reduction of active compound yielded, hence resulting in lower mortality rate on the mention part and concentration. The fluctuation of this mortality could also be caused by interference of glycosides bounded in the bioactive compounds in *Tridax* plant. It was reported that the glycosylation of compounds decreases the efficiency in some of the chemical activities in a plant (Evans et al. 1996; Li et al. 2009). Other than that, the effectiveness of plant extract in causing mortality is depending on the intake of the compound trough ingestion.

This study concludes that *T. procumbens* were found to have larvicidal properties against *Aedes* larvae. Larvicide from plant derivative is known to be harmless to the environment, and it is safer to use (Rahuman et al. 2008). Environment safety is a priority to us, thus an insecticide does not need to contribute high mortality to the target organism but should be environment friendly (Anupam et al. 2011) Extracts from this plant also need to be further tested in the field to test its efficacy against the dengue vectors in different seasons and conditions in comparison to the synthetic larvicide. Further study and analysis can be conducted on *Tridax procumbens* to improve its larvicidal properties on *Aedes spp.* larvae.

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