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Larvicidal activity of *Wedelia chinensis* (Asteraceae) plant extracts against *Aedes aegypti* and *Culex quinquefasciatus*

KEYWORDS: Larvicidal activity, Wedelia chinensis, GC-MS analysis

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ABSTRACT *Larvicidal activity of Methanol, Ethyl acetate, Hexane and Acetone extracts of Wedelia chinensis (Asteraceae), were tested against the early fourth instar larvae of Aedes aegypti L. and Culex quinquefasciatus. The larval mortality was observed after 24 h of exposure different solvents of leaf and stem extracts of W. chinensis were subjected to dose–response bioassay for larvicidal activity against the larvae of A. aegypti and C. quinquefasciatus. The numbers of dead larvae were counted after 24 h of exposure, and the percentage of mortality was reported from the average of five replicates. However, at the end of 24 h, the selected test samples methanol and acetone solvent have higher toxic potential and hexane and ethyl acetate have less toxic potential to the mosquito larvae. Of the various ratios tested, W. chinensis can be applied as an ideal potential larvicide against A. aegypti and C. quinquefasciatus. This is an ideal ecofriendly approach for the control of the dengue vector, A. aegypti, and the lymphatic filariasis vector, C. quinquefasciatus.*

Introduction
Malaria is the world's most dreadful tropical disease. Mosquito-borne diseases are endemic in more than over 100 countries, causing mortality of nearly two million people every year, and at least one million children die of such diseases each year, leaving as many as 2,100 million people at risk around the world (Kager 2002). As reported recently, 406 million Indians were at risk of stable Plasmodium falciparum transmission in 2007 with an uncertainty point estimate of 101.5 million clinical cases (95% CI 31.0–187.0 million cases; (Hay et al., 2010). In India, Malaria is still the most important cause of morbidity and mortality with approximately two to three million new cases arising every year (Sharma 2003). Dengue fever incidence has increased fourfold since 1970 and nearly half the world's population is now at risk. In 1990, almost 30% of the world population, 1.5 billion people, lived in regions where the estimated risk of dengue transmission was greater than 50% (Hales et al. 2002

The wound healing efficacy of ethanolic leaf extract of Wedelia chinensis was evaluated in excision, incision and dead space wound models. Its ethanolic extract was found to possess significant wound healing activity, which was evidenced by decrease in the period of epithelialization, increase in the rate of wound contraction, skin breaking strength, granulation tissue dry weight, and its breaking strength (Verma et al., 2008). Therefore, the aim of this study was to investigate the mosquito larvicidal activity of the petroleum ether extracts of five plant species from India. The present study was an attempt to assess the larvicidal activity of leaf and stem extracts against A. aegypti and C. quinquefasciatus.

Materials and methods
The Leaves and stem of Wedelia chinensis (Asteraceae) were collected from Auxillium College campus, Katpadi, Vellore in November 2013 and the taxonomic identification was made by Ms. Isabella Rosaline, S., M.Sc., M.Phil., (Ph.D.,) Department of Botany, Auxilium College, Katpadi, Vellore.

Mosquito culture
Larvae of A. aegypti and C. quinquefasciatus were collected from stagnant water area of Vellore (12° 56'23"N, 79° 14'23"E) and identified in Zonal Entomological Research Centre, Vellore, Tamil Nadu, India. To start the colony, the larvae were kept in plastic and enamel trays containing tap water. They were maintained and reared in the laboratory as per the method of (Kamaraj et al., 2009). The larvicidal activity was assessed by the procedure of WHO (1996) with some modifications (Rahuman et al., 2000).

Preparation of plant extracts
The fresh leaves and stem of Wedelia chinensis were thoroughly washed to remove debris and the earth remains. From these the stem were divested and chopped into bits and allowed to dry for 7-14 days under shade at the room temperature. The 300g of dried leaves and stem were weighed using an electronic weighing balance and powdered by using electrical grinder. The powder was soaked in hexane (1200ml), acetone (1200ml), ethyl acetate (1200ml) and methanol (1200ml). The powder soaked in solvents for three days. The extracts were filtered through a Buchner funnel with What man's number 1 filter paper. Then the filtered sample was poured into a soxlet apparatus (boiling point range 100°C). After 2 hours the filter paper was concentrated at room temperature until oily paste formed and kept at cool dry place for further use.

Larvicidal bioassay
One gram of crude extract was first dissolved in 100 mL of respective solvent (stock solution). From the stock solution, 500 ppm was prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05% in the final test solution. The larvicidal activity was assessed by the procedure of WHO (1996) with some modification and as per the method of (Rahuman et al., 2000). For bioassay test, larvae were taken in five batches of 20 in 249 mL of water and 1.0 mL of the desired plant extract concentration. The control was set up with respective solvent and polysorbate 80. The numbers of dead larvae were counted

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after 24 h of exposure and the percentage of mortality was reported from the average of five replicates. The experimental media in which 100% mortality of larvae occurs alone were selected for dose- response bioassay.

Dose-response bioassay
From the stock solution, different concentrations ranging from 31.25 to 500 ppm were prepared. Based on the preliminary screening results, different solvents of leaf and stem extracts of W. chinensis were subjected to dose–response bioassay for larvicidal activity against the larvae of A. aegypti and C. quinquefasciatus. The numbers of dead larvae were counted after

24 h of exposure and the percentage of mortality was reported from the average of five replicates. However, at the end of 24 h, the selected test samples methanol and acetone solvent have higher toxic potential and hexane and ethyl acetate have less toxic potential to the mosquito larvae.

Statistical analysis
The average larval mortality data were subjected to probit analysis for calculating LC_{50} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit which calculated by using the software developed by (Reddy et al., 1992). Results with $p<0.05$ were considered to be statistically significant.

Results and discussion
The larvicidal activity of solvent plant extracts is often attributed to the complex mixture of active compounds. The preliminary screening is a good means of evaluation of the potential larvicidal activity of plants popularly used for this purpose. Larvicidal activity of different solvent extracts of Wedelia chinensis are noted and presented in (Table 1and 2).

The larvicidal activity of W.chinensis leaf extract against A.aegypti revealed the lowest level of mortality (30.0%) for hexane and the highest level (57.2%) for methanol groups. In the control group there was no mortality. The difference between the means of the five groups were statistically significant. The larvicidal activity of W. chinensis stem extract against A.aegypti revealed the lowest level of mortality (24.6%) for hexane and the highest level (48.4%) for methanol group. There was no mortality in the control group. The larvicidal activity of W.chinensis leaf extract against C. quinquefasciatus revealed the lowest level of mortality (24.8%) for hexane and the highest level (62.0%) for methanol groups. There was no mortality in the control group. The difference between the means of five groups was statistically significant. The larvicidal activity of W. chinensis stem extract against C. quinquefasciatus revealed the lowest level (23.2%) for hexane group and the highest level (53.8%) for methanol group. There was no mortality in the control group. The difference between the means of five groups was statistically significant.

GC- MS analysis
In the results pretaining to the GC-MS analysis, three compounds were detected in the solvent methanol leaf extract of Wedelia chinensis. The major chemical constituent was identified as Tridecanoic acid peak area (21.29) by comparison of mass spectral data and retention times (Fig 1). The other constituents present in the solvent methanol leaf extract were N-hexadecanoic acid (22.15), L-(+)-Ascorbic acid 2, 6-Di hexadecanoate (24.74). The GC- MS analysis of acetone, three compounds were deteced in the solvent acteone leaf extract of Wedelia chinensis. The major chemical constituent was identified as C(14A)-Homo-27-norgammacer-13-EN-21-ol, 3-methoxy-(3, Alpha, 21 BET) peak area (8.15), 2-chloroethyl linoleate (18.12) and 1-phenanthrenemethanol, 1,2,3,4,4a,9,10,10a octahydro-1-4a-dimethyl (25.66).

Discussion
The larvicidal activity of W.chinensis leaf extract against A.aegypti revealed the lowest level of mortality (30.0%) for hexane and the highest level (57.2%) for methanol groups. In the control group there was no mortality. The larvicidal activity of W. chinensis stem extract against A.aegypti revealed the lowest level of mortality (24.6%) for hexane and the highest level (48.4%) for methanol group. The larvicidal activity of W. chinensis stem extract against C. quinquefasciatus revealed the lowest level of mortality (23.2%) and the highest level (53.8%) for methanol group. The difference between these values was statistically significant. Studies focusing on the effect of Wedelia chinensis against larvicidal activity have not been carried out so far. In conclusion, an attempt has been made to evaluate the role of plant extracts in mosquito larvicidal activity. The results reported in this study open the possibility of further investigations on the efficacy of the larvicidal properties of natural product extracts.

Conclusion
In conclusion, an attempt has been made to evaluate the role of medicinal plant extracts' larvicidal bioassay against A. aegypti and C. quinquefasciatus activity. The results reported in this study open the possibility for further investigations of the efficacy of larvicidal properties of natural product extracts. The isolation and urification of crude extract of leaf and seed methanol extracts of Wedelia chinensis are in progress.

Table1. Larvicidal activity of leaf and stem extracts of Wedelia chinensis against A.aegypti

Extract	Group	Mortality Level (%)		F
		Mean	SD	
Leaf	Hexane	30.0	24.3	4.3**
	Ethylacetate	39.6	23.6	
	Acetone	54.0	30.3	
	Methanol	57.2	31.2	
	Distilled water (Control)	0	0	
Stem	Hexane	24.6	19.2	3.6*
	Ethylacetate	33.8	25.0	
	Acetone	39.6	25.4	
	Methanol	48.4	27.7	
	Distilled water (Control)	0	0	

Table2. Larvicidal activity of leaf and stem extracts of Wedelia chinensis against C. quinquefasciatus

Extract	Group	Mortality Level (%)		F
		Mean	SD	
Leaf	Hexane	24.8	21.3	6.4**
	Ethylacetate	48.6	23.4	
	Acetone	55.8	26.4	
	Methanol	62.0	29.7	
	Distilled water (Control)	0	0	
Stem	Hexane	23.2	19.7	4.8**
	Ethylacetate	34.6	19.9	
	Acetone	40.0	21.9	
	Methanol	53.8	29.2	
	Distilled water (Control)	0	0	

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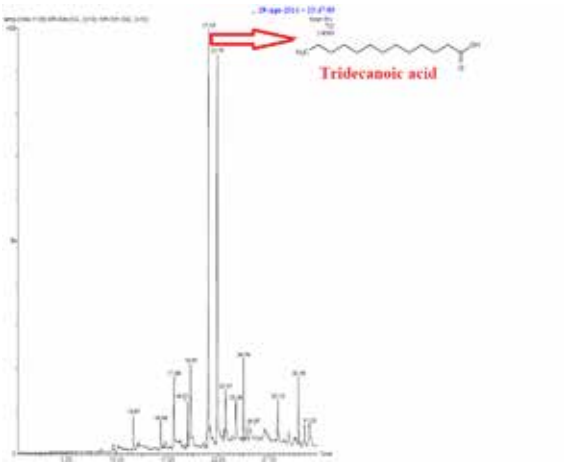
Figure1. GC – MS analysis of methanol leaf extract of Wedelia chinensis



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