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Research Article

Larvicidal effect of *Mundulea sericea (Leguminosaea)* plant extract against *Aedes aegypti* (L.) (Diptera: *Culicidae*)

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Background: *Aedes aegypti* is the main carrier for viruses that cause dengue, dengue hemorrhagic and yellow fevers. Insecticide use to control this vector has led to the development of mosquito resistance, environmental pollution, and undesirable effects on non-target organisms. Consequently, interest in insecticides of natural origin, specifically plant-derived products, continues to receive close attention.

Objective: To evaluate organic extracts of *Mundulea sericea* root bark and seedpods for efficacy against *Aedes aegypti* larvae.

Methodology: The plant parts were separated, dried and ground into fine powder and successively extracted using selected solvents. The dried extracts were dissolved in dimethylsulphoxide (DMSO) to prepare four to five different concentrations of each extract. The larvae were then exposed to concentrations ranging from 50 to 3000 parts per million (ppm) of the extracts in an aqueous medium for 24 hrs at 25 - 30 °C.

Results: Hexane extract of the root bark displayed the most remarkable potential, with an LC_{50} of 130 ppm, followed by 180 ppm for methanol and 450 ppm for dichloromethane. Comparatively, methanol extracts from the root bark had significantly higher activity than that of the seedpods.

Conclusion: These findings suggest that bioactivity of phytochemicals from *M. sericea* plant varies significantly depending on solvent used in extraction. Moreover, root bark extracts were more than seedpod extracts. Overall, hexane extracts of the root bark have the potential of being developed as larvicides for mosquito control.

Key words: Leguminasaea, *Mundulea sericea*, *Aedes aegypti*, larvicide

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1. Introduction

The medical importance of mosquitoes as vectors for the transmission of serious diseases that cause morbidity, mortality, economic loss, and social disruption such as malaria, lymphatic filariasis, and viral diseases is well recorded (Becker et al, 2003). *Aedes aegypti*, the main carrier for viruses that cause dengue and dengue hemorrhagic and yellow fevers, is found majorly in the tropics and subtropics. There is no effective vaccine against dengue, and thus the only way of significantly lowering the incidence of this disease is through mosquito control (Malavige et al, 2004). Chemical measures were first tried, but they failed since their overuse led to disruption of natural biological control systems and outbreak of new insect species. In addition, use of insecticides led to the development of mosquito resistance, environmental pollution, and undesirable effect on non-target organisms (Brown, 1986). In a bid to resolve these problems, interest in insecticides of natural origin, specifically plant-derived products has recently received close attention.

Several studies have emphasized the importance of research and development of herbal substances for controlling mosquitoes (Shaala et al, 2005). Their results may vary, but natural plant products may be a possible alternative to synthetic substances, as they are effective and compatible with human and animal life and the environment (Chaithong et al, 2006).

The genus Mundulea consists of about 15 species, widespread throughout Africa, Madagascar, Mauritius, India, Sri Lanka and Papua New Guinea. Only a single species, *Mundulea sericea*, is found in Southern Africa. This species occurs in South Africa, Botswana, Namibia and Angola, north to tropical Africa, and east to Madagascar, India, Sri Lanka and Papua New Guinea (Watt and Breyer-Brandwick, 1962).

Mundulea sericea is one of the commonest fish poisons where both bark and seeds are used (Neuwinger, 2004). In addition, the Chinese used *M. sericea* to control tobacco budworm *Heliothis virescens* (Lepidopteriae: Noctuidae) (Yoshida and Toscano, 1994).

The toxic principal of the plant is rotenone, an isoflavonoid (Vedcourt and Trump, 1969). The rotenoids deguelin and tephrosin are the potent active principles which have been isolated from extracts of *M. sericea* (Luyengi et al, 1994). Deguelin is a natural plant-derived rotenoid, most commonly used as an insecticide in Africa and South America (Udeani et al, 1997). Rotenoids from the bark of *M. sericea* have been commercially used as insecticide. These chemical compounds in the bark, leaves and seed are the active compounds responsible for the fish poison. It is reported that the strength varies geographically (Watt and Breyer-Brandwick, 1962).

The current study involved extraction and evaluation of root bark and seedpod of *M. sericea* for larvicidal activities on *Aedes aegypt.*

2. Materials and methods

2.1 Plant collection and preparation

The roots and the seed pods of *M. sericea* were collected from Kericho District and identified by a Mr. Mathenge, a Taxonomist at the East Africa Herbarium, National Museums of Kenya, where a voucher specimen was deposited (Voucher no: MS 002). The plant parts were separated, dried and ground into fine powder using a laboratory grinding mill. Successive extraction was carried out on the plant material, starting with hexane followed by dichloromethane, and finally methanol. Before use, the samples were stored in a refrigerator at -4 °C until use in the larvicidal bioassays.

2.2 Mosquito colony and maintenance

Laboratory bred mosquito larvae were used. An *Ae. aegypti* mosquito colony was set up at Kenya Medical Research Institute (KEMRI). Eggs for its initiation were obtained from International Centre for Insect Physiology and Entomology (ICIPE). The mosquitoes were maintained in the insectary at temperatures of 30 - 37 °C. Ground dry yeast was regularly provided to the larvae in a pan containing tap water. Adults were kept in cages and glucose was provided using cotton wool soaked in 6% glucose solution. Anaesthetized BALB/c mice or hamsters were regularly introduced into the cage for mosquitoes to acquire blood required for egg development.

2.3 Larvicidal bioassays

Larvicidal activity of the crude extracts was evaluated as per protocol described earlier (WHO, 1981). Dry extracts of hexane, dichloromethane, and methanol were dissolved in dimethylsulphoxide (DMSO) to prepare graded series of concentrations. Batches of 10 early 4th instar larvae of *Ae. Aegypti* were transferred in 25 ml of water to a 500 ml bowl containing 249 ml of distilled water and 1ml of the varying concentrations of each plant extract. Three replicate tests were carried out simultaneously, with a final total of 30 larvae for each concentration. The toxicity of each plant extract was evaluated with four to five concentrations yielding a range of 0 - 100% mortality. Negative controls received DMSO-distilled water, while the untreated larvae were maintained in water only. These bioassays were performed at 25 - 30 °C.

After treatment, the larvae were considered dead if, at the end of 24 hrs, they showed no sign of swimming movements even after gentle touching with a glass rod, as described in the World Health Organization's technical report series (WHO, 1981). The dead larvae in the three replicates were combined and expressed as a sum mortality of each concentration.

2.4 Data analysis:

The analysis program Probit (Finney, 1971) was used in the determination of LC_{50}, LC_{95}, and the diagnostic concentration at LC_{99} in 24 hrs. SPSS version 12 was used in determining the probit values.

3. Results and discussion

In this study, early 4th instar larvae of *Ae. aegypti*, under laboratory conditions were subjected to rising concentrations of solutions of both polar and non-polar extracts derived from *M. sericea* (**Table 1**). After treatment with varying concentrations of methanol (80

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– 1550 ppm), dichloromethane (50 – 1540 ppm) and hexane (50-410 ppm) root bark extracts, the larval mortality increased from 26.7 - 100%, 6.7 - 100%, 10.0 - 93.3%, respectively (**Table 1**).

Table 1: Larvicidal activity of hexane, dichloromethane and methanolic extracts derived from *M. sericea* root bark against 4th instar larvae of *Ae. Aegypti*

Extract (ppm)	% mortality	Larvicidal activity (ppm)			
		LD_{50}	LD_{95}	LD_{99}	χ^2
Methanol					
1550	100	180	590	750	30.5
500	90				
300	83.3				
100	70				
80	26.7				
0	0				
Dichloromethane					
1540	100	450	860	1030	27.6
800	73.3				
600	80				
500	86.7				
80	6.7				
50	0				
0	0				
Hexane					
410	93.3	130	390	500	41.9
200	53.3				
100	73.3				
80	70				
50	10				
0	0				

Similarly, methanol extracts of the root bark and the seedpods were correspondingly compared for activity. Mortalities of 26.7 - 100% and 0.0 - 73.3% were observed, for doses of 80 - 1550 ppm and 50 - 772 ppm, respectively (**Table 2**). In the untreated control groups, no mortality was observed within 24 hrs and the larvae developed into pupae and then adults within 48 - 72 hrs. Among the root bark extracts, hexane extracts displayed more remarkable larvicidal potential than methanol and dichloromethane. Their respective LC_{50}, LC_{95} and LC_{99} values in ppm were 130, 390, and 500 (hexane), 180, 590, and 750 (methanol), and 450, 860 and 1030 (dichloromethane). Methanol extracts of the seedpods exhibited LC_{50}, LC_{95} and LC_{99} values of 650, 1090 and 1270 ppm (**Table 2**), respectively.

Table 2: Larvicidal actvity of methanolic extracts of the root bark and seedpod of *M. sericea* species against 4th instar larvae

Extract (ppm)	% mortality	Larvicidal activity (ppm)			
		LD_{50}	LD_{95}	LD_{99}	χ^2
Root bark					
1550	100	180	590	750	30.5
500	90				
300	83.3				
100	70				
80	26.7				
0	0				
Seed pod					
772	46.7	650	1090	1270	19.19
600	73				
500	23				
80	0				
50	0				
0	0				

In addition, hexane, methanol, and dichloromethane extracts exhibited χ^2 of 41.9, 30.5 and 27.6 (**Table 1**), in that order. χ^2 for the seedpod was 19.19 (**Table 2**). These chi-square values, however, indicate that the differences between the observed and expected mortalities in the experimental groups were highly significant. This could be attributed to unaccountable variations amongst the replicates such as individuals competing for the same resources. Therefore, the mortality values could change without the risk of invalidating the results (Lee et al, 2001; Preisler, 1988), as observed in bioassays where food is added (Preisler et al, 1990), as was the case in this study.

In the current study, levels of active constituents in each extract may be responsible for the observed differences in their larvicidal potential against *Ae. aegypti*. The hexane extract contains non-polar chemical compounds, dichloromethane extract contain mid-polar compounds and methanol extract contain polar compounds. Therefore, the high activity seen in the hexane extract was due to non-polar compounds and low activity observed in methanol extract was due to polar compounds.

Several studies on larvicidal potential of natural products for controlling *Aedes* mosquitoes have been carried out. However, varying results were obtained. Previous studies showed that ethanol extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica*, two members of the family Meliaceae, were found to have lethal effects on *Ae. aegypti* larvae, with LC_{50} values ranging from 0.017 to 0.034 g % (Wandscheer et al., 2004). Moreover, ethanolic extracts derived from three species of the Piperaceae (pepper) family, *Piper longum*, *P. ribesoides* and *P. sarmentosum*

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had toxic effect on *Ae. aegypti* 4th instar larvae. Their LC_{50} values ranged from 2.23 to 8.13 ppm (Chaithong et al, 2006). The insecticidal activity of 11 extracts from nine South American medicinal plants were studied using the *Ae. aegypti* larvicidal assay. Eight of the 11 plant extracts studied showed toxicity against the *Ae. aegypti* larvae (LC_{50} < 500 µg/ml). The dichloromethane extracts of *Abuta grandifolia* and *Minthostachys setosa* demonstrated high larvicidal activity, the most active being the dichloromethane extract of *A. grandifolia*, with an LC_{50} = 2.6 µg/ml (LC_{100} = 8.1 µg/ml). On the other hand, the dichloromethane extract of *M. setosa* was quite potent against *A. aegypti* larvae showing an LC_{50}=9.2 µg/ml (Lyege *et al*., 2010). Larvicidal activity of ethyl acetate, butanol, and petroleum ether extracts of five species of Euphorbiaceae plants, *Jatropha curcas*, *Pedilanthus tithymaloides*, *Phyllanthus amarus*, *Euphorbia hirta*, and *Euphorbia tirucalli*, were tested against the early fourth instar larvae of *Aedes aegypti*. The larval mortality was observed after 24 hrs of exposure. All extracts showed low larvicidal effects; however, the highest larval mortality was found in petroleum ether extract. The LC_{50} value of petroleum ether extracts of *J. curcas*, *P.tithymaloides*, *P. amarus*, *E. hirta*, and *E. tirucalli* were 8.79, 55.26, 90.92, 272.36, and 4.25 ppm, respectively, against *A. Aegypti* (Rodrigues et al, 2005) .

4. Conclusion

Our findings indicate that the toxic components responsible for larvicidal effect in the plant are concentrated in the hexane extract of root bark. Results from our study revealed that the larvicidal potential of *M. sericea* extracts is comparable to previous studies on natural products. Nonetheless, further studies for the isolation and identification of bioactive compounds especially in the hexane extract would be useful in developing new types of mosquito larvicides. Moreover, further studies need to be performed to recognize the mode of action between the extract and mosquito larvae.

Conflict of Interest declaration

The authors declare no conflict of interest

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