Open Science Repository Pharmaceutics

Larvicidal efficacy of *Mundulea sericea (Leguminosaea)* plant extract against *Anopheles gambiae (Giles)* and *Culex quinqefasciatus (Say)* (Diptera: *Culicidae*)

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Background: *Anopheles gambiae* is the main carrier for parasites that cause malaria and filariasis as well as viruses that cause yellow fever, dengue fever, dengue haemorrhagic fever, chikungunya and encephalitis. *Culex quinqefasciatus* on the other hand is the main vector for parasites that cause filariasis and the virus that cause encephalitis. Insecticide use to control these vectors has led to the development of mosquito resistance, environmental pollution, and undesirable effects on non-target organisms. Consequently, interest in insecticides of natural origin, particularly plant derived products, continues to receive much attention.

Objective: To evaluate organic extracts of *Mundulea sericea* stem bark and leaves for efficacy against *Anopheles gambiae* and *Culex quinqefasciatus* 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 25 to 50,000 parts per million (ppm) of the extracts in an aqueous medium for 24 hrs at 25 - 30 °C.

Results: Ethanol extract of the stem-bark displayed the most remarkable potential, with an LC90 of 188 ppm and 210 ppm for *An. gambiae* and *Cx. cuinqefasciatus* respectively. Leaf water extract displayed the highest LC50 of 45,000 ppm on *Cx.quinqefasciatus* and 9,000 ppm on *An. gambiae*. Comparatively, ethanol extracts from the stem-bark had significantly higher activity than that of the leaves.

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

Key words: Leguminasaea, *Mundulea sericea*, *Anopheles gambiae*, *Culex quinqefasciatus*, larvicide

1. Introduction

The economic importance of mosquitoes as vectors of pathogens that cause serious diseases in human beings is well documented. The diseases such as malaria, lymphatic filariasis, and viral diseases are known to cause high morbidity, mortality, economic loss, and social disruption (Becker *et al*, 2003). *Anopheles gambiae,* the main carrier for parasites that cause malaria and filariasis as well as viruses that cause yellow fever, dengue fever, dengue haemorrhagic fever, chikungunya and encephalitis; and, *Culex quinqefasciatus,* the principal vector for parasites that cause filariasis and the viruses that cause encephalitis are found majorly in the tropics and sub-tropics. There are no effective vaccines against most of the diseases that they cause, and thus the only way of significantly lowering the incidence of these diseases is through mosquito control (Malavige *et al*, 2004). Chemical measures though very effective initially have failed as their overuse has led to disruption of natural biological control systems and outbreak of new insect species. Moreover, the use of insecticides has 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 botanicals has recently received close attention (Shaala *et al*, 2005). Current research findings indicate that 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 insecticides. 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 stem bark and leaves of *M. sericea* for larvicidal activities on *Anopheles gambiae* and *Culex quinqefasdciatus.*

2. Materials and methods

2.1 Plant collection and preparation

The stem-barks and the leaves of *M. sericea* were collected from Bamba in Kilifi County and Busia in Busia County, identified by taxonomists at the Kenya Medical Research Institute. 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 ethanol then water. The samples were stored in a refrigerator at -4 °C until their use in the larvicidal bioassays.

2.2 Mosquito colony and maintenance

Laboratory bred mosquito larvae were used. *Anopheles gambiae* pink-eyed mosquito colony was set up at Kenya Medical Research Institute (KEMRI). Eggs for its initiation were obtained from the Centre for Global Health Research in Kisumu. The mosquitoes were maintained in the insectary at temperatures of 30- 37°C, 80% relative humidity (RH) and 12:12 hours Light to Darkness (L:D) photoperiod. Ground dry yeast was regularly provided to the larvae in a pan containing tap water. Adults were kept in cages and sucrose was provided using cotton wool soaked in 6% sucrose solution. Anaesthetized Swiss-albino mice anaesthetized with Pentabarbitone Sodium (Sagatal^{R}) were used to supplement the female's diet and to initiate vitellogenesis. *Cx. quinqefasciatus* stock was collected from the adult strains existing in Ngummo area of Nairobi, Kenya using light traps. They were identified by taxonomists at KEMRI to distinguish them from *Cx. pipiens* (L.) which is also common in the area. The females were fed on blood meals, allowed to oviposit and then discarded. The eggs were used to establish the experimental colony. The larvae were fed on ground Weetabix^{R} while females were provided with 5% sucrose solution and blood from Swiss-albino mice anaesthetized with Pentabarbitone 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 ethanol and water were dissolved in dimethylsulphoxide (DMSO) to prepare graded series of concentrations. Batches of 25 late 3rd instar larvae of *An. gambiae* and *Cx. quinqefasciatus* were transferred in 25 ml of water to a 500 ml bowl containing 200 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 25 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 LC50, LC95, and the diagnostic concentration at LC99 in 24 hrs. SPSS version 12 was used in determining the probit values.

3. Results and discussion

In this study, late 3rd instar larvae of *An. gambiaee* and *Cx. quinqefasciatus*, under laboratory conditions were subjected to rising concentrations of solutions of both ethanol and water extracts derived from *M. sericea* stem bark (**Table 1a** and **b**). After treatment with varying concentrations of ethanol stem bark extracts, the larval mortality rate of *An. gambiae* increased from 2±4.9802SE to 25±4.9802SE while that of *Cx. quinqefasciatus* larvae increased from 1±5.4201SE to 22±5.5401SE. After treatment with varying concentrations of water stem bark extracts, the larval mortality rate of *An. gambiae* increased from 1±4.7075SE to 25±4.7075SE while that of *Cx. quinqefasciatus* larvae increased from 1±2.4702SE to 15±2.4702SE (**Table 1a** and **b**).

Table 1(a): Larvicidal activity of ethanol and water extracts derived from *M. sericea* stem bark against 3rd instar larvae of *An. gambiae*

Extract (ppm)	**% mortality**	**Larvicidal activity (ppm)**			
		LD50	**LD90**	**r**	**F**
Ethanol					
250	100	188	900	0.962	52.108
150	88				
100	60				
50	32				
25	8				
0	0				
Water					
450	100	200	3820	0.926	26.111
350	92				
250	88				
100	28				
50	4				
0	0				

Table 1(b): Larvicidal activity of ethanol and water extracts derived from *M. sericea* stem bark against 3rd instar larvae of *Cx. quinqefasciatus*

Extract (ppm)	**% mortality**	**Larvicidal activity (ppm)**			
		LD50	**LD90**	**r**	**F**
Ethanol					
250	88	210	875	0.877	15.303
150	84				
100	80				
50	48				
25	4				
0	0				
Water					
450	64	500	7,190	0.918	23.451
350	40				

250	12
100	4
50	0
0	0

Similarly, ethanol and water extracts of the leaves were correspondingly compared for activity. After treatment with varying concentrations of ethanol leaf extracts, the larval mortality rate of *An. gambiae* increased from 10±6.1801SE to 25±6.1801SE while that of *Cx. quinqefasciatus* larvae increased from 1±0.9210SE to 5±0.9210SE. After treatment with varying concentrations of water leaf extracts, the larval mortality rate of *An. gambiae* increased from 0±0.7280SE to 25±7.28005SE while that of *Cx. quinqefasciatus* larvae increased from 3±1.701SE to 8±1.701SE (**Table 2a** and **b**). 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 stem bark extracts, ethanol extracts displayed more remarkable larvicidal potential than water. The respective LC50 and LC90 values of ethanol stem bark extracts in ppm were 900 and 1,876 respectively (*An. gambiae*); 875 and 2,100 respectively (*Cx. quinqefasciatus*). For water stem bark extracts the LC50 and LC90 in ppm were 200 and 3,820 respectively (*An. gambiae*); 500 and 7,190 respectively (*Cx. quinqefasciatus*). The respective LC50 and LC90 values of ethanol leaf extracts in ppm were 900 and 6,100 respectively (*An. gambiae*); 2,500 and 5,500 respectively (*Cx. quinqefasciatus*). For water leaf extracts the LC50 and LC90 in ppm were 9,000 and 18,800 respectively (*An. gambiae*).There was no significant correlation between the mortality rate of *Cx. quinqefasciatus* larvae and the leaf water extract concentration. (**Table 2a and b**).

Table 2(a): Larvicidal activity of ethanol and water extracts derived from *M. sericea* leaves against 3rd instar larvae of *An. gambiae*

Extract (ppm)	**% mortality**	**Larvicidal activity (ppm)**			
		LD50	**LD90**	**r**	**F**
Ethanol					
8,000	100	900	6,100	0.752	5.600
6,000	100				
4,000	96				
2,000	84				
1,000	40				
0	0				
Water					
20,000	100	9,000	18,800	0.886	16.501
16,000	96				
12,000	92				
8,000	72				
4,000	0				
2,000	0				
0	0				

In **Table 2(b)**: Larvicidal activity of ethanol and water extracts derived from *M. sericea* leaves against 3rd instar larvae of *Cx. quinqefasciatus*

Extract (ppm)	**% mortality**	**Larvicidal activity (ppm)**			
		LD50	**LD90**	**r**	**F**
Ethanol					
8,000	20	25,000	55,000	0.966	57.652
6,000	12				
4,000	8				
2,000	8				
1,000	4				
0	0				
Water					
20,000	32	45,000	92,400	0.446	2.609
16,000	20				
12,000	28				
8,000	40				
4,000	32				
2,000	12				
0	0				

In addition, the r and F values of stem bark ethanol and water extracts did not show significant differences in mean mortality rates in the experimental mosquito larvae investigated (**Table 1a and b**). The r and F values for the leaf extracts did not show significant differences in mean mortality rates of the mosquito larvae investigated except in the case of *M. sericia* leaf water extract where there was significant difference in mean mortality rates (**Table 2a and b**). This could be attributed to poor solubility of active compounds from the leaves in water or a possible metabolic degradation of the active compounds in water or rapid excretion of the compounds by the larvae reducing the rate of mortality rate (Tsukamoto and Casida, 1967).

In the current study, levels of active constituents in each extract may be responsible for the observed differences in their larvicidal potential against *An. gambiae* and *Cx. quinqefasciatus* larvae. The ethanol extract contains both polar and non-polar chemical compounds while water contains polar compounds only. Therefore, the high activity seen in the ethanol extract was due to the non-polar compounds and low activity observed in water extract was due to polar compounds.

Several studies on larvicidal potential of natural products for controlling 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 various mosquito larvae (Wandscheer *et al*., 2004). Moreover, ethanolic extracts derived from three species of the Piperaceae (pepper) family, *Piper longum, P. ribesoides* and *P. sarmentosum* had toxic effect on different mosquito larvae (Chaithong *et al*, 2006). The insecticidal activity of 11 extracts from nine South American medicinal plants was studied using different mosquito larvicidal assays. Eight of the 11 plant extracts studied showed toxicity against the larvae. The ichloromethane extracts of *Abuta*

grandifolia and *Minthostachys setosa* demonstrated high larvicidal activity, the most active being the dichloromethane extract of *A. grandifolia*. On the other hand, the dichloromethane extract of *M. setosa* was quite potent against larvae (Lyege *et al*., 2010). Larvicidal activity of ethyl acetate, butanol, and petroleum ether extracts of five species of Euphorbiaceae plants, *Jatropha curcas*, *Pedilanthus ithymaloides*, *Phyllanthus amarus*, *Euphorbia hirta*, and *Euphorbia tirucalli*, were found to induce larval mortality after 24 hrs of exposure. (Rodrigues *et al*, 2005).

4. Conclusion

Our findings indicate that the toxic components responsible for larvicidal effect in the plant are concentrated in the ethanol extract of stem 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 ethanol 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

Acknowledgements

The authors acknowledge and appreciate the support given by the staff members of Center for Biotechnology Research and Development (CBRD), Kenya Medical Research Institute (KEMRI). This research project was financed through KEMRI internal grants.

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