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Mosquitocidal activity of *Millettia pachycarpa* on the larvae and eggs of *Aedis aegypti*

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ABSTRACT

Millettia pachycarpa Benth. (family Fabaceae) is a well-known medicinal plant in the traditional systems of Chinese and the Mizo tribes of India. One of its many uses is as an insecticide. The extract of the root bark was examined for larvicidal and ovicidal activities against the dengue vector mosquito Aedis aegypti Linn (Diptera: Culicidae). The early fourth instar larvae were treated with serial concentrations of the plant extract (viz. 6.25, 12.5, 25, 50, 100 and 200 mg/L) continuously for 24 h. Data indicate that the plant extract exerted profound lethal activity (LC_{50} = 98.47 ppm at 24 h) on the larvae. For evaluation of the ovicidal activity, concentration was increased until when there was complete inhibition of egg hatching. Mean percent hatchability of the eggs was noted at different time intervals from the freshly laid eggs up to 24 h old. At high concentration (200 mg/L) of the plant extract, there was complete inhibition of egg hatching (100% non-hatchability). The observation also indicated that the percent hatchability was inversely proportional to the concentration of the extract, and directly proportional to the age of the eggs. Therefore, the extract of M. pachycarpa root bark exhibited significant mosquitocidal activity against A. aegypti.

Keywords: *Aedis aegypti*, egg, larvicide, LC_{50}, *Millettia pachycarpa*, ovicidal.

INTRODUCTION

Mosquitoes are the primary vectors for the most dreadful and fatal diseases such as dengue, malaria, yellow fever, filariasis, Japanese encephalitis and chickungunya. The vector-borne diseases caused by different species of mosquitoes constitute an unsurpassed health problem all over the world, remaining as the leading cause of mortality. *Aedes (Stegomyia) aegypti* Linn is a dipteran mosquito that is disseminated throughout the urban areas of the world with an immense medical importance being a vector for dengue in Asia, and for dengue and yellow fever in Africa and the Americas [1]. According to current estimate around 2.5 billion people are at now risk of dengue, which become the most rapidly spreading mosquito-borne viral disease. Further, the disease incidence has increased thirtyfold in the last 50 years [2].

There have been recurrent outbreaks of dengue fever in India associated with rapid increase and spread of *A. aegypti*, particularly in major towns and cities. In Mizoram, the remotest north-eastern state of India, there is also a record of drastic increase in the number of this mosquito, which was otherwise unrecognized a decade ago. In fact, the principle urban places such as Aizawl, Champhai and Kolasib are recently noted to be the highest in the incidence of *A. aegypti* among the north eastern states of India [3].

The most reliable strategy of minimizing the incidence of mosquito-borne diseases is to eradicate and control the mosquito vectors, which is performed principally by systematic treatment of the breeding places through a combination of environmental management and application of larvicides that do not harm other organisms in the environment [4]. However, the most commonly used larvicides are now in dire questions of their sustained dissemination due to their potential environmental pollution, and hazards to human health and other non-target organisms, particularly when profusely applied where there are epidemics. The situation is further compounded by the fact that the most rampant mosquitoes have developed resistance to all conventional larvicides [5]. These inevitable dilemmas have prompted renewed interest in the search and development of better or alternate vector control strategies that destroy the insects over a wide range, with minimal effect to non-target organisms and the environment. Therefore, traditional practices using indigenous plants turn out to be a major potential alternative approach. *Millettia pachycarpa* Benth. (family Fabaceae) is a leguminous perennial climbing tree endemic to south-east Asia, where it is acclaimed with a wide range of medicinal applications in various traditional practices. The root bark, seed and leaf are commonly used as a blood tonic, treatment of infertility, fish stupefying, anticancer and insecticidal agent [6,7]. A large number of bioactvie compounds have been identified from it, of which isoflavones such as erysenegalensein E, isoerysenegalensein E, 6,8-diprenylorobol, millewanins G and H, furowanin A and B, and auriculasin were all demonstrated to have antiestrogenic activity [8,9]. Following the traditional usage of the Mizo tribes of north-east India, the extract of the root bark was demonstrated to have significant anthelmintic activity against the cestode Raillietina echinobothrida [7,10,11]. The present investigation is an attempt to assess the larvicidal and ovicidal activities on A. aegypti.

MATERIALS AND METHODS

Plant material

The fresh roots of M. pachycarpa were collected from the nearby forest of Aizawl (which occupies the coordinate of 23.73° North and 92.72° East, and situated at an altitude of 3,340 feet above sea level), Mizoram, India. Identification and authentication of the plant material was reported elsewhere [10].

Preparation of the plant extract

The root barks were peeled off, thoroughly washed with deionized water, cut into small pieces, and dried in a hot air oven at 50ºC. The dried parts were crushed to fine powder and then refluxed with ethanol (100g/L) for 8 h at 60ºC, as described earlier [7,10]. The solution obtained was filtered through Whatman filter paper (No. 1) and the solution was evaporated to complete dryness at 50ºC. The crude extract was obtained as a deep brown powdered material, which was then refrigerated at 4ºC until further use. The net yield from such extraction was 7.07%. 1 h prior to experimental assay, varying concentrations of the extract, viz. 6.25, 12.5, 25, 50, 100 and 200 mg/L, were prepared by dissolving in double-distilled water, supplemented with 1% dimethylsulfoxide (DMSO).

Larvicidal assay

Collection of *A. aegypti* eggs, rearing of larvae, data recording and assessment of larvicidal activity were performed as per the guidelines of WHO [12]. To synchronize and promote hatching, larval food (3:1 mixture of biscuit and yeast powder) was added to the culture medium 24 h before adding the eggs. Experiments were conducted for 24 h at the temperature of 25±2°C in an automated glass-chambered incubator. Homogenous population of late third or early fourth instars (5 days old and ~5 mm in length) were obtained five to seven days later. For bioassay test, larvae were taken in 5 batches, each consisting of 25 individuals, and introduced in disposable containers containing the desired concentration of the plant extract. Different concentrations of the test samples were used. Control medium consisted of only distilled water with 1% DMSO. All the test solutions were maintained at 7 cm depth. Each test was repeated three times. The numbers of dead larvae were counted after 24 h of exposure, and the percentage mortality was recorded from the average of five replicates. Death was confirmed when larvae failed to respond upon probing with a needle in the siphon or the cervical region. Percentage mortality was corrected using Abbot's formula [13]:

$$\text{Mortality (\%)} = \frac{X - Y}{Y}\ 100$$

where X is percentage survival of the control group, and Y is that of the treated group.

Ovicidal assay

Ovicidal activity was determined using the method of Su and Mulla [14]. The egg raft of *A. aegypti* was introduced into nine glass vials. Of these nine vials, eight were each filled with test solution of 6.25, 12.5, 25, 50, 100 and 200 mg/L, and one was filled with deionised water supplemented with 1% DMSO that served as a control. The egg raft/eggs containing approximately 100 eggs were laid (within 4 h, most of the egg rafts/eggs were laid) in the culture medium and routinely collected at 4 h interval up to 24 h. Egg raft/eggs were selected from the different time intervals at random and individually transferred to the different concentration of extract for 3 h. After treatment, the egg raft/eggs from each concentration were individually transferred to distilled water cups for hatching. The total number of hatched and unhatched eggs was assessed after counting the eggs under microscope. Each test was replicated in five and repeated three times.

Data analyses

Data from all replicates were pooled for analysis. LC_{50} was calculated from a log dosage-probit mortality regression line based on the method of Finney, using computer software programme, BioStat 2008 version 5.5, AnalystSoft Inc., Vancouver, Canada. Data were presented as mean±standard deviation. Comparison of the efficacy was estimated using Student's *t*-test and significant level was considered at $P < 0.05$.

RESULTS

The efficacy of *M. pachycarpa* root bark extract on the fourth instar larvae of *A. aegypti* is presented in Table 1. The larvicidal effect of the plant extract was clearly dependent on the concentration of the extracts. All the larvae maintained in the control medium survived for 24 h, thus, no mortality for the control experiment. Even at the lowest concentration (6.25 mg/L) tested, the plant extract caused mortality as high as 26.32%, and mortality at higher concentrations such as 12.5, 25, 50 and 100 mg/L were 41.65, 54.84, 76.75 and 92.52, respectively. Complete mortality (100%) was observed on the larvae only at the highest

concentration, 200 mg/L. Therefore, the lethal concentration (LC_{50}) of the extract was determined to be 98.47, with the lower and upper confidence limits (95%) of 85.59 and 107.17, respectively.

Table 1. Lethal activity of an ethanolic extract of *Millettia pachycarpa* root bark at different concentrations against fourth instar larvae of *Aedes aegypti.*

Incubation medium	Concentration (mg/L)	Mortality (%)	LC_{50} (ppm)	95% Confidence limits	
				Lower	Upper
M. pachycarpa extract	0 (Control)	0	98.47 ± 0.62	85.59 ± 0.83	107.17 ± 0.42
	6.25	26.32 ± 0.22*			
	12.5	41.65 ± 0.51*			
	25	54.84 ± 0.87*			
	50	76.75 ± 0.94*			
	100	92.52 ± 0.40*			
	200	100.00 ± 0.62*			

*n =5; * P < 0.05 using Student's t-test in comparison to the control.*

Hatchability of the eggs of *A. aegypti* after exposure to different concentrations of *M. pachycarpa* root bark extract is shown in Table 2. The result clearly indicates that the higher level of ovicidal activity by the plant extract was observed in the early stage of egg development. In the control medium (water supplemented with DMSO) the eggs showed full (100%) hatchability at every age. At the highest concentration (200 mg/L), no egg of any age was detected to develop, thus, no hatchability. Inhibition of egg hatching was clearly proportional to the concentration of the plant extract. From the results also, it is quite apparent that susceptibility to the plant extract decreases with age, as the younger age groups of egg rafts/eggs showed a poor hatchability when exposed to higher concentrations of the extract, and older age groups of egg rafts/eggs showed a high hatchability rate when exposed to lower concentrations of the extract.

Table 2. Inhibitory activity of an extract of *Millettia pachycarpa* root bark on the eggs of *Aedes aegypti.*

Age of eggs (h)	Percentage of egg hatching Concentration of the plant extract (mg/L)						
	0	6.25	12.5	25	50	100	200
0-4	100	58.4	31.6	NH	NH	NH	NH
4-8	100	75.7	48.2	22.5	NH	NH	NH
8-12	100	83.1	63.9	44.3	28.5	NH	NH
12-16	100	92.8	86.0	62.7	38.2	NH	NH
16-20	100	100	95.3	78.2	56.7	26.8	NH
20-24	100	100	100	82.7	67.4	43.5	NH

n = 5; NH = No hatchability (i.e. 100% mortality).

DISCUSSION

Results from the present study clearly provided evidence that the extract of *M. pachycarpa* contains a mosquitocidal component. A number of plants have been investigated for their mosquitocidal properties. *Ageratum conyzoides*, *Anacardium occidentalis*, *Argemone mexicana*, *Azadirachta indica*, *Carapa guianensis*, *Cassia fistula*, *Copaifera langsdorffii*, *Cymbopogon winterianus*, *C. citratus*, *Jatropha curcus* and *Solenostemma argel* reportedly have significant potential in the control of *Culex* and *Anopheles* species [15-20].

Several plants are also documented to be active against *A. aegypti*. Gusmão *et al.* [22] reported that the ethanol extracts of *Derris urucu* were effective against the fourth instar larvae with LC_{50}

of 17.6 ppm. A closely related species of the present investigation, the methanol extract of *Millettia dura* seed caused significant larvicidal activity (LC_{50} of 3.5 ppm) on the second instar larvae [23]. The essential oils of *Lippia sidoides* exhibited larvicidal effects more potent than temephos used in Brazil [24, 25]. Similarly, essential oils from *L. multiflora* exhibited larvicidal and ovicidal activity [27]. Thirteen oils from 41 plants were demonstrated to induce 100% mortality after 24 h, or even after shorter periods; the best oils indicated LC_{50} ranging between 1 and 101.3 ppm against *A. aegypti*, between 9.7 and 101.4 ppm for *Anopheles stephensi* and between 1 and 50.2 ppm for *C. quinquefasciatus* [28]. The essential oil of the stalks and leaves of *Croton argyrophylloides*, *C. nepetaefolius*, *C. sonderianus* and *C. zehntneri* showed significant mortality [29]. Morais *et al*. [30] also showed that methyleugenol and alpha-copaene of *C. nepetaefolius* indicated LC_{50} of 84 ppm; α-pinene and β-pinene of *C. argyrophyloides* indicated LC_{50} of 102 ppm; and α-pinene, β-phelandrene, and trans-caryophyllene of *C. sonderianus* indicated LC_{50} of 104 ppm, and that of *C. zenhtneri* was 28 ppm.

Derris elliptica showed LC_{50} values between 11.2 and 18.84 ppm against *A. aegypti*, *C. quinquefasciatus*, *Anopheles dirus* and *Mansonia uniformis* [21]. Shalaan *et al.* [31] showed high activity of *Callitris glaucophylla* against *A. aegypti* and *Culex annulirostris*. *Curcuma zedoaria* rhizome volatile oil exhibited pronounced lethal activity against the fourth instar larvae of *A. aegypti* with an LC_{50} of 33.45 ppm [32]. To the essential oil from the seeds of *Zanthoxylum armatum*, *C. quinquefasciatus* was the most sensitive (LC_{50} of 49 ppm) followed by *A. aegypti* (LC_{50} of 54 ppm) and *A. stephensi* (LC_{50} of 58 ppm) [33]. Eleven of the 84 Brazilian plant extracts studied showed significant activities against the larvae of *A. aegypti*, with best results for the extracts of *Annona crassiflora* (root bark, LC_{50} of 0.71 ppm; root wood, LC_{50} of 8.94 ppm) and *A. glabra* (seed, LC_{50} of 0.06 ppm) [34]. The 24 h LC_{50} concentration of the methanol, benzene and acetone extract of *C. fistula* were observed at 10.69, 18.27 and 23.95 ppm, respectively [35]. Significantly high larvicidal activities were demonstrated for the ethanolic extracts of *P. longum*, white *P. nigrum* and black *P. nigrum* (LC_{50} values were 0.248, 0.356, and 0.405 ppm, respectively) [36].

CONCLUSION

Of its many traditional uses *M. Pachycarpa* is a well-known insecticidal plant in Chinese and Mizo practices. The present investigation shows that the extract of the root bark in deed exhibited significant ($P < 0.05$) lethal activity and egg hatching inhibition upon the mosquito *Aedes aegypti*. The efficacy of the plant extract is comparable to those of the many of the well-established insecticidal plants. Therefore, the present study presents the rationale for the traditional usage of the plant, and warrants further investigation on the mode of action of the plant on the mosquito, and the active principle involved.

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