Mosquito Larvicidal Activity Of *Trichilia Emetica* Vahl. (Meliaceae)

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Various plant parts from *Trichilia emetica* of Tanzania, were screened for larvicidal activity against *Anopheles gambiae* and *Culex quinquefasciatus*. The pericarp methanol extract exhibited LC_{50} values of 3.5 and 10.8 ppm against *An. gambiae* and *Cx. quinquefasciatus* larvae, respectively, while the values were 6.5 and 4.3 ppm respectively, for the seed coat extract. Other plant parts had low larvicidal mortality. An extract of the seed kernel of *Azadirachta indica* A. Juss. was less active than *T. emetica*, exhibiting LC_{50} values of 177.8 and 398.1 ppm, against *An. gambiae* and *Cx. quinquefasciatus*, respectively. From these preliminary findings *T. emetica* may be regarded as a promising larvicide, for the control of malaria and other mosquito-transmitted diseases.

Key Words: *Trichilia emetica, Azadirachta indica,* larvicides, *Anopheles gambiae, Culex quinquefasciatus.*

INTRODUCTION

Mosquitoes of the genera *Anopheles*, *Aedes* and *Culex* are responsible for most of the human bites. Through bites they act as vectors of diseases such as malaria, lymphatic filariasis, yellow and dengue hemorrhagic fever. Mosquito control methods include the use of repellents for personal protection, insecticides and larvicides to interrupt their life cycle.

In an effort to search for new, effective and environmentally friendly mosquito control agents, it was decided to screen Tanzanian plants for larvicidal activity. This paper describes an investigation done on *Trichilia emetica* Vahl. (Syn. *T. roka* Chiov.) (Meliaceae). This plant is distributed in almost all regions of Tanzania [1], where it is used for medicinal, edible and cosmetic purposes. The leaf is used as a purgative and emetic, the fruit pericarp is used as emetic [1,2] and the root and stem bark are used for the treatment of persistent barrenness [3]. The seed, which is black, is almost covered with a red aril; and when soaked in warm water and squeezed, it yields an edible milk-like liquid. Oil from the seed kernel is used for cosmetic purpose and for soap manufacture [1,2]. Previous studies on this plant dealt mainly with its anti-feedant activity against the Southern Army Worm [4,5] and anti-yeast activity [6]. This study aimed at investigating the larvicidal potential of *T. emetica* for mosquito control. An extensively studied related insecticidal plant, *Azadirachta indica* A. Juss. (Neem) from the Meliaceae, was also evaluated for larvicidal activity for comparison purpose.

MATERIALS AND METHODS

Plant materials

Various parts of *Trichilia emetica* (roots, stem bark, leaves and fruits) were collected from Kyela district, in Mbeya region, Tanzania. All parts were collected in August 1994, with the exception of fruits, which were collected in February 1995. Fruits of *Azadirachta indica* were collected in February 1995 from trees grown in the Muhimbili Medical Centre area in Dar es Salaam, Tanzania. The plants were authenticated by the Institute of Traditional Medicine, Muhimbili University College of Health Sciences (MUCHS). Voucher specimens are deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, MUCHS.

Preparation and extraction of plant materials

Fruit walls (pericarps) were separated from seeds, then all plant materials were dried in the shade, and further drying was done in a hot-air oven at 60° C, just before further processing. After drying, seed coats were separated from the kernel, then all plant materials were ground in a hammer mill. Each powdered material (50 g) was extracted with 200 ml of methanol by maceration at room temperature for 24 h, with occasional shaking. Extracts were decanted, filtered and the solvent was evaporated to dryness, with a rotary evaporator, under vacuum at a temperature of 50° C. In the case of *T. emetica* separate extracts were obtained from the root, stem bark, leaves, pericarp, seed coat and kernel. For *A. indica* only the kernel was extracted.

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Preparation of extracts for larvicidal assay

Extracts were initially dissolved in ethanol to make a stock solution with extract concentration of 40 mg/ml. This was then diluted with an appropriate volume of distilled water to make the required test concentrations. Initially extracts were tested at 200 ppm (μg/ml), and for the determination of concentrations which caused 50% larvae mortality (LC_{50}), five-fold serial dilutions were prepared for extracts exhibiting at least 50% mortality at 200 ppm. Therefore the final test concentrations ranged from 200 ppm to 0.32 ppm.

Larvicidal assay

Second to fourth instar *Anopheles gambiae* and *Culex quinquefasciatus* larvae were used in this study. *Anopheles gambiae* larvae were obtained from a laboratory-reared strain of the mosquito, maintained in the insectary unit of the Department of Parasitology and Entomology, Institute of Public Health (IPH), MUCHS. *Culex quinquefasciatus* larvae were collected from water pools within the Muhimbili Medical Centre, in Dar-es-Salaam and were identified by the staff of the Department of Parasitology and Entomology, IPH. They were allowed to get adapted to laboratory conditions for 24 h prior to performing larvicidal tests. Both larvae species were utilized in tests involving serial concentrations, but for preliminary screening at 200 ppm, only *Cx. quinquefasciatus* larvae were used, due to their ready availability. Tests were performed at a temperature of 26 - 29° C and a relative humidity of about 70%. The larvicidal assay involved exposure of at least ten larvae to the extract solution (100 ml) in a beaker. Mortality was assessed after an exposure period of 24 h. A beaker containing the same volume of 0.5% v/v aqueous ethanol served as a control. Tests were performed in duplicate and were repeated two more times on different days, so that a total of at least sixty larvae were exposed to each concentration and control. Percent larvae mortality was determined after correction for control mortality using the Abbott's formula as shown below:

$$\% \text{ Mortality} = \frac{[\text{Sample \% mortality} - \text{Control \% mortality}]}{[100 - \text{Control \% mortality}]} \times 100$$

Tests in which control mortality exceeded 10% were discarded. The LC_{50} values (24 h) were determined by probit analysis, using a log-probit paper.

RESULTS AND DISCUSSION

Table 1 shows the results of the larvicidal evaluation of methanol extracts of various parts of *Trichilia emetica* and the seed kernel of *Azadirachta indica*. The results indicate that *T. emetica* extracts possess larvicidal activity against both *An. gambiae* and *Cx. quinquefasciatus* mosquito larvae. However, the fruit exhibited stronger activity than other parts. The pericarp and seed coat extracts caused 100% mortality on *Cx. quinquefasciatus* at 200 ppm, while the seed kernel and other plant parts exhibited, only 15 - 25% larval mortality. The LC_{50} values obtained for the pericarp and seed coat extracts against both types of mosquito larvae are indicated in Table 1. In general *Anopheles gambiae* larvae were more sensitive to the pericarp than the seed coat extract, while *Cx. quinquefasciatus* larvae were slightly more sensitive to the seed coat extract.

TABLE 1: LARVICIDAL ACTIVITY OF *TRICHILIA EMETICA* AND *AZADIRACHTA INDICA* EXTRACTS

Type of Extract	*Cx. quinquefasciatus*		*An. Gambiae*
	% mortality (200 ppm)	LC_{50} (ppm)	LC_{50} (ppm)
T. emetica:			
Root	15	-[a]	-
Stem bark	15	-	-
Leaf	25	-	-
Pericarp	100	10.8	3.5
Seed coat	100	4.3	6.5
Seed kernel	15	-	-
A. indica:			
Seed kernel	55	398.1	177.8

Key: [a] Mortality at 200 ppm was low, hence LC_{50} values were not determined.

The constituents responsible for larvicidal effect have not yet been identified, but *T. emetica* has been reported, previously, to contain limonoids, such as trichilins A – F, sendanin and others [4-6]. These are tetranortriterpenoids, some of which were found to be responsible for plant's antifeedant activity against the Southern Army Worm [4,5]. The observed mosquito larvicidal effect could possibly be due to these compounds. Limonoids, are responsible for the insecticidal, growth inhibitory, feeding deterrent, mosquito larvicidal and repellent effect of various Meliaceae species, such as *Azadirachta indica* and *Melia volkensii* [7-9], as well as plants in the Rutaceae [9]. A crude methanol extract of the kernel of *A. indica* fruit was included in this study for comparison purposes, since the kernel is considered to be the most active part against insects. However, its larvicidal effect was inferior when compared with that of the pericarp and seed coat of *T. emetica*. This could be due to the fact that neem derivatives do not kill insects directly, but rather cause behavioral and physiological stress, which eventually leads to mortality [10]. The compounds in *T. emetica*, on the other hand, may be having a direct killing effect on larvae.

The larvicidal activity of *T. emetica* is located in a regenerative plant part, the fruit. This makes the fruit a suitable candidate for development of new larvicides, since its exploitation would not endanger the plant. It is also interesting to note that the active parts of the fruit are the pericarp and seed coat, which are usually discarded when the seed is taken for the preparation of oil for soap manufacture and cosmetic purpose. However, the seed coat has been reported to be extremely toxic [1,2]. Hence it may not be of high priority for development, where necessary, it could be used in water collections, which are not used for domestic purposes or animal consumption. Further work needs to be done to assess the safety of the pericarp and to isolate the active constituents of this part of the fruit.

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