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SHORT COMMUNICATION

LARVICIDAL ACTIVITY OF MYRICOIDINE FROM *CLERODENDRUM MYRICOIDES*

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ABSTRACT. Chromatographic separation of a methanolic extract of *Clerodendrum myricoides* led to the isolation of myricoidine (**1**) whose structure was elucidated using IR, NMR and melting point. The crude fractions as well as **1** were subjected to larvicidal tests using the second instar larvae of *Anopheles gambiae* (malaria vector) at concentrations of 100, 75, 50, and 25 ppm. The crude extract and the compound were found to be active with LC_{50} values of 14 and 9 ppm, respectively.

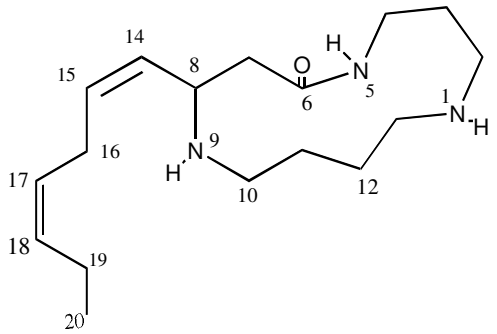
KEY WORDS: *Clerodendrum myricoides, Anopheles gambiae, S*econd instar larvae, Myricoidine

INTRODUCTION

Malaria remains a major public health problem in Africa; it contributes to 1.5-3 million deaths of which one million are due to cerebral malaria [1].

Following the evolution of mosquito strains that are resistant to insecticides, combined with a rapid increase in mosquito population, there is need to develop alternative environmentally friendly and cost-effective tools for control of the mosquito population. One way of doing this is by disrupting the life cycle of the insect at an early stage before it transmits the disease. As part of an on-going research on malaria, *Clerodendrum myricoides* (Verbenaceae) was screened for larvicidal activity. The plant is known for ethnomedical uses in most African countries [2-7]. The extract of *Clerodendrum myricoides* is also known to have antibacterial, antiviral and antifungal [3], antimalarial [7, 8], antidiarrhoeal [9], and antifeedant [10] activities.

This study led to the isolation of myricoidine (**1**), which was investigated for larvicidal activity against *Anopheles gambiae.*



(**1**)

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EXPERIMENTAL

The melting point was determined using a Stuart melting point apparatus with a thermometer range of 0-360 ^{o}C and was uncorrected. Infrared spectrum was run on an IR-408 spectrometer, for the compound isolated as KBr disc. ^{1}H and ^{13}C NMR analyses were done using a Bruker WM (250 MHz) NMR spectrometer. The solvent used was deuteriated chloroform with TMS as the internal standard. Column chromatography was done on silica gel (size 0.063-0.200 mm). The *Anopheles gambiae* eggs were obtained from International Centre for Insect Physiology and Ecology (ICIPE). The hatched larvae were fed on Tetraminbaby food (proteins 47.0%, oil 8.0%, fibre 2.0%, ash 10.0%, and moisture 6.0%). The roots of *Clerodendrum myricoides* were collected from Uasin-Gishu district, which is 320 km West of Nairobi, Kenya. The staff of Botany Department Herbarium, Moi University, where a voucher specimen was deposited, identified the plant.

Solvent extraction and chromatography

The whole roots of the plant were chopped into small pieces, air dried at room temperature for two weeks, then ground into a powder. The powder (1.5 kg) was soaked in cold methanol for 4 days to extract the compounds. The resulting dark yellow filtrate was concentrated under reduced pressure using a rotatory evaporator to a dark brown semi-solid, 10 g of which was chromatographed over silica gel, eluting with increasing amounts of ethyl acetate in n-hexane. Eluted fractions were monitored by thin layer chromatography using n-hexane and ethyl acetate (7:3) as developing solvent. The column was washed with pure methanol. Eight fractions were obtained. The first three fractions were n-hexane extract containing fats and were discarded. The fractions 4-8, upon combination and concentration, gave a volume of 10 mL. This was subjected to further column chromatography for purification leading to the isolation of **1**, which was recrystallized using acetone and methanol mixture (1:1).

Larvicidal test

Mosquito larvicidal tests were done using the second instar larvae of *Anopheles gambiae.* The *Anopheles gambiae* eggs were hatched in one litre of distilled water and incubated at 40 ^{o}C. The oven temperature of 40 ^{o}C ensured that water containing the larvae was maintained at the required temperature of 32 ^{o}C. The larvae were fed using Tetraminbaby complete food.

Stock solutions whose concentration was 100 ppm were prepared for both the crude extract and compound **1.** The stock solutions were prepared by dissolving 10 mg of each in 100 mL of water and acetone mixture (60:40) that was used to dissolve the extracts. Each concentration was applied at a rate of 20 mL into petri dishes containing 10 larvae each in triplicate. The control experiment contained acetone and water (60:40) and 10 larvae only. The effect of the crude extract and compound **1** on the larvae was observed every 24 hours for a period of 10 days. The number of dead larvae was recorded for each observation.

The mean mortality (%) of each concentration for the three dishes for both the crude extract and compound **1** were calculated and a graph of mean mortality verses concentrations was plotted which was used to obtain their respective LC_{50} values.

RESULTS AND DISCUSSION

Structural elucidication

Myricoidine (**1**) was isolated as white crystals, m.p. 127-129 ^{o}C. The spectroscopic data (IR and NMR) conformed to data of the same compound isolated by Bashwira [11] from *C. myricoides* in Zaire. However this is the first report of the compound isolated from the Kenyan *C. myricoides*.

Larvicidal test

The crude extract from which myricoidine (**1**) was isolated had an LC_{50} value of 14 ppm. This larvicidal activity was lower than that of **1**, which had LC_{50} value of 9 ppm. Therefore larvicidal activity of **1** is enhanced through isolation and purification. It seems that synergistic effects of some other components in the crude extract lower the overall larvicidal activity.

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