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Larvicidal Activity of 2, 5-dihydroxy - 3 - methyl -1, 4 - benzoquinone

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Separation of ethyl acetate extract from *Embelia schimperi* using chromatographic techniques led to isolation of 2, 5-dihydroxy-3-methyl-1,4-benzoquinone (**1**). The its crude extract and compound (**1**), exhibited larvicidal activity against *Anopheles gambiae* larvae with LC_{50} of 3.496 ppm and 1.82 ppm respectively. Based on spectroscopic (^{1}H NMR, ^{13}C NMR, IR and MS) data and melting point of 117ºC-118ºC, compound (**1**) was identified to be as 2, 5-dihydroxy - 3 - methyl -1, 4 - Benzoquinone.

Key words: Embelia schimperi, malaria, mortality, mosquito larva, 2, 5-dihydroxy - 3 - methyl-1, 4-benzoquinone.

Introduction

Malaria, derived from Italian meaning 'bad air' has plagued man for probably more than 100 million years. An estimate of 500million cases of malaria occur in the world each year; and 1 to 2 million of these individuals especially children below 5years die (WHO, 1998).

Among all infectious diseases, malaria may rank between 6th to 7th causes of death, (Sachs and Malaney 2002). About 2-5 billion people are at risk of contracting the disease, out of these, 15 million are in Kenya.

Mosquitoes are vectors of malaria. Anopheles gambiae is a species of mosquito which is the most efficient malaria vector. As with other mosquito species, they lay eggs on water, which hatch into larvae. The larvae are aquatic and develop through a series of four (4) stages called instars into pupae. The pupae then develop into adults (Buchel, 1983).

The aquatic habitats of the mosquito larvae are varied and include permanent and temporary water bodies. Species are found in fresh pools or mashes often associated with floating vegetation. Some prefer sunlit areas while others don't. Large water bodies of open water do not usually provide breeding places, though larvae may live in pools or pockets of still water near the margins of such habitats. Low attitudes and high temperatures support mosquito population resulting in a year round transmission of malaria.

The sites available for the larvae help determine the control measures in particular areas, for example engineering works connected with irrigation, drainage and other forms

of water control may have an effect on aquatic habitats, encourage or discourage the growth of mosque\to population (Awala, *et al.*, 1998).

After the discovery of malaria transmission and the importance of *Anopheles gambiae* mosquito as the vector, efforts have been geared towards development of chemicals and other agents for elimination of the vector. With introduction of compounds like DDT and synthetic pyrethroids, there was considerable spectacular suppression of malaria cases. By and large, the total number of people freed from malaria after rising from 3.7 million in 1961 to 720 million in 1971 fell to 436 million in 1976 (Scientific Working Group on Malaria, 2003). The disease was almost eradicated in places like Cuba, Europe and U.S.A. Despite all these successes, mosquito resistance to DDT and other organochlorine insecticides were reported in many species of mosquitoes, (David and Colin 1991). Other alternative insecticides like organophosphates and carbonates are expensive and ineffective. Furthermore, DDT is toxic to non target organisms, is non biodegradable and accumulates in the food chain.

Organic compounds from plants such as nicotine and pyrethrins have been used. Elsewhere, a mixture of hydrocarbons or derived organic compounds and synthetic organic ovicides, were also known as potent insecticides (Mayunga, 2002)

Mosquito resistance to common insecticides, ignorance of mosquito bionomics, poverty and lack of involvement in control programs has hampered the effective control of the disease.

Emphasis needs to be placed in vector control once more. Plants that are traditionally used in control of insects in grain storage or as insect repellants could be one such good source for new safe, biodegradable and renewable insecticides. It is in this regard that *Embelia, schimperi* was the subject of this investigation.

Plant description

Embelia schimperi belongs to the family *Myrsinaceae* and is a highland shrub with evergreen simple alternate leaves. The plant grows naturally in a mixture of loam, peat and sandy soil. The *Myrsinaceae* family is distributed all over the world. In Kenya, the species is found around Mt. Kenya, Ngong Hills, Kakamega forest and Western slopes of the Mau ranges in Kericho District, (Kokwaro, 1993). Many communities in Kenya use the species as an herb. The Maasai and the Kipsigis tribes living in the Rift Valley province of Kenya use it to deworm both humans and animals in cleaning wounds and as a disinfectant (Bogh *et al.*, 1996).

The aim of the research was to extract and evaluate the extracts from *E. schimperi* for mosquito larvicidal activity.

Materials and Methods

Collection of plant parts

Stem bark of the herbal plant *Embelia schimperi* was collected from the Western slopes of Mau Ranges in Kericho District which is about 300km west of Nairobi. The plant was identified by the herbarium staff of the Department of Botany, Moi University,

where a voucher specimen (No. Ker 02 05 001) was deposited. The collected plant parts were air-dried for 10 days, chopped into small pieces and ground into fine powder.

Extraction and isolation of 2, 5-dihydroxy - 3 - methyl -1, 4 - Benzoquinone

The compound was extracted and isolated for use in larvicidal assay according to a procedure by Ooko et al., (2008)

Larvicidal assay

Eggs of *Anophele gambiae* mosquito were obtained from a standard colony at International Centre for insect Physiology and Ecology (ICIPE) were incubated in de-ionized water at a temperature of 37ºC–40ºC. The eggs hatched within a period of 24 h.

A quantity of 20 mg of compound **1** was dissolved separately in 200 ml of de-ionized water to make 100 ppm stock solution. Subsequently, 75 ppm, 50 ppm and 25 ppm of the same fraction were made by serial dilution of the stock solution using the de-ionized water. Each concentration was divided into three portions of 20 ml each and put in separate sample vials. Concentrations of the crude extract were also prepared using the same procedure.

Commercially available pyrethrin was used as a standard larvicide. It was prepared by dissolving 0.5 ml of the larvicide in 1 liter of water (as per the manufacturer's specification.

Twenty (20) young larvae of *Anopheles gambiae*, identified as the 2^{nd} instars were introduced into the sample vials containing compound 1 and the crude extract with concentrations ranging from 25 ppm to 100 ppm. To each sample vial, three granules of yeast were added. The yeast granules served as food for the larvae.

Two control experiments were set up. The first one contained larvae and yeast alone, while the second one contained larvae, yeast and pyrethrum larvicide. All the experiments were done in triplicates.

The sample vials were then taken back into the incubator while maintaining the same solution temperature of between 37ºC and 40ºC, (Cheesbough, 2000).

Dead larvae were counted and removed from the solution at 24 h intervals for 72 h. Any larvae that was still active in the test drug media by the end of 72 h was considered to be resistant to the test drugs. In the study, a dead larva was defined as one that did not swim to the surface for air and lay still at the bottom of the sample vial or one that floated on the surface of the solution and ceased to swim.

The data was recorded as cumulative mortality for each of the concentrations for each set of vials. The percentage mortality was arrived at by dividing the number of dead larvae by the total number of larvae multiplied 100.

The data obtained was used to draw graphs of mean mortality versus concentration. These graphs were used to determine the LC_{50}, the concentration of the test drug needed to kill 50% of the larvae.

Graphs of mean mortality (%) against respective concentrations (ppm) were plotted using Microsoft excel data base for both the crude and compound **1**. Best lines were obtained using the same data base. The equation of the lines are generally given as;

$y=mx+c$, where y is the mean mortality, m is the gradient of the line; x is the concentration of the crude and the isolated compound.

The lethal concentration values were obtained from the above graphs at mean mortality of 50%. The value obtained for each compounds was substituted in place of y in each linear equation and the concentration value x was obtained by calculation. The value of x gives the lethal concentration of the crude extract and the isolates at 50% mortality (LC_{50}). The results are summarized in table II.

Results and Discussions

Crude extract attained 100% mortality at all concentration within 24 h while 100 ppm of compound **1** caused 100% mortality after 48 h. Embelin (**2**) had been subjected to this test earlier using the larvae of *Aedes aegypti* and were found to disrupt the process metamorphosis before causing mortality (Kiprono, 1997).

Table II: LC_{50} values of the Crude and *2, 5-dihydroxy - 3 - methyl -1, 4 – Benzoquinone* (**1**)

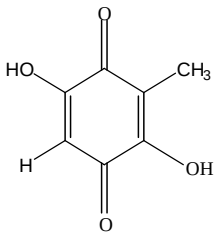
Crude and isolated compound	LC_{50}	P-values	R-square
Crude	1.824 ppm	$4.23x10^{-4}$	0.9248
*Compound **1***	3.496 ppm	$2.79x10^{-6}$	0.9123
pyrethrin	1.22 ppm		

From the LC_{50} values the crude has a higher activity of LC_{50} = 1.824ppm against the pure compound (**1**) with LC_{50} = 3.496 ppm.

A regression analysis using SPSS database showed that the effect of an increase of the drug concentration on mortality was significant. This explained by Pearson's correlation coefficient falling below 0.005 ($p<0.005$) as well as R-square values output being very close to unity for both the crude and compound (**1**).

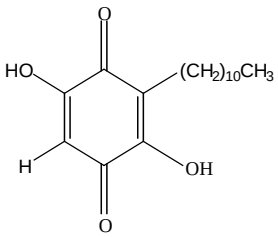
High activity of crude plant extracts is not new in science. It had been noted that insects respond to a profile of plant chemicals rather than an individual chemical. For example crude extracts of the neem tree (*Azidarichta indica)*- a Maliaceae - are more toxic to army worms than azidarichtin, the principal active compound (Hodgson, 1990).

Fig. 1. Structures of *2, 5-dihydroxy - 3 - methyl -1, 4 - Benzoquinone* (**1**) and embelin (**2**)



M/e = 154.022

(**1**)



M/e = 294.183

(**2**)

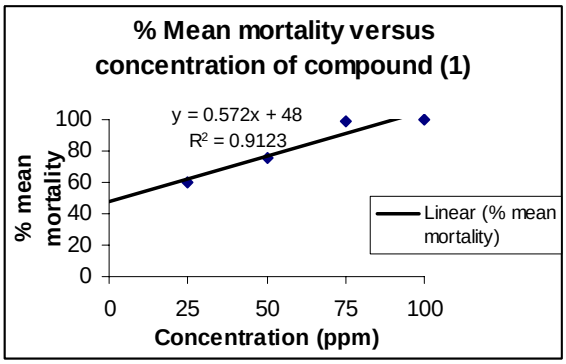


Fig. 1: % Mean mortality caused by compound (**1**)

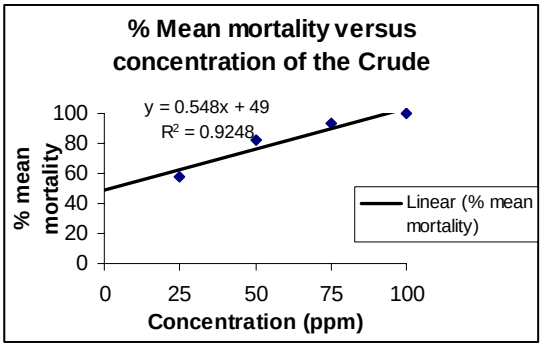


Fig. 2: % Mean mortality caused by crude extract

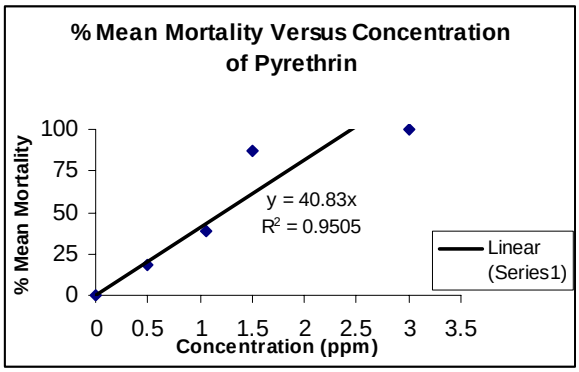


Fig. 3: % Mean mortality caused by pyrethrin

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