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Larvicidal activity of *Combretum collinum* Fresen against *Aedes aegypti*

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Despite of the fact that dengue outbreaks in the tropics have been worldwide for over 200 years, it is still the most important mosquito transmitted viral disease affecting man[1]. *Aedes aegypti* (Diptera: Culicidae) is an arbovirus vector responsible for yellow fever in central and south America and in west Africa. It is also the vector for dengue hemorrhagic fever (DHF), endemic to south-east Asia, the Pacific Islands, Africa and the Americas[1].

It is estimated that 2.5 billion people are currently at risk for dengue fever (DF), DHF, and dengue shock syndrome (DSS)[1]. The size and spread of the dengue pandemic, the unpredictability of the epidemic occurrences and the circulation of virulent and non-virulent strains make DHF/DSS a model for emerging infectious disease. Despite of this challenge, the development of dengue virus vaccines is still a long way to be of any use due to several obstacles[2].

The threat by DHF/DSS is magnified by the aggressive day-time feeding habits of *Ae. aegypti* making it harder to control compared to *Anopheles* mosquitoes[3]. Current mosquito control strategies include the use of aerial sprays (toxicants) repellents, larvicides, insecticides as well as mosquito nets for personal protection. However, aerial toxicants against *Ae. aegypti* are ineffective because this mosquito is highly domesticated and many adults rest indoors in hidden places such as closets. The only effective way of reducing mosquito densities to a level where dengue epidemics do not occur is by attacking the larval breeding places[4]. Unfortunately, many of these methods are not readily affordable and available in the developing world setting[5]. Therefore, in response to the urgent need for new, affordable, effective and environment-friendly mosquito control agents; we screened a Ugandan medicinal plant of the family—Combretaceae (*Combretum collinum* Fresen) for mosquito larvicidal activity against *Ae. aegypti. C. collinum* also classified as an aromatic plant is found growing in the wild (western, eastern and northern Uganda) with different tribes of Uganda referring to it according to their own dialect—for instance, in the Lusoga dialect, it is called *Mukoola omukali*[6].

Following is a review of some of the medicinal uses of *C. collinum*. In Bulamogi County (eastern Uganda), a decoction from the roots is drunk for the treatment of diarrhoea, sterility and pyomyositis. Furthermore, an infusion from the roots is orally given to delivering women to enhance labour; to children with hydrocele and patients with gonorrhoea to treat the latter conditions respectively[6]. Root powder from *C. collinum* may be drunk as 'tea'; or mixed with bathing water for bath by epileptic patients. Alternatively, the root powder

may be incorporated in petroleum jelly base for topical application in the treatment of epilepsy[6].

While some medicinal properties of this plant in Uganda have previously been studied (isolated classes of constituents of Combretaceae family were shown to include triterpenoids and tannins[7]) there are no formal reports of its larvicidal activity against *Ae. aegypti,* hence the need for this study. To demonstrate the potential of *C. collinun* as a mosquito larvicide against *Ae. aegypti,* Neemazal F extract from *Azadirachta indica* A. Juss (Neem) from Meliaceae family was used as a larvicidal compound[8] against which the action of *C. collinun* was compared.

Fresh *C. collinum* shoot bark was collected from plants growing naturally in Oculoi village, 15 km from Soroti district (in eastern Uganda) during the second week of April 2002. The plant was authenticated by a botanist from Makerere University, Kampala, Uganda. The shoot bark of *C. collinum* was washed, chopped and air-dried away from direct sunshine to a constant weight for six months. The dried plant material was pounded into a powder using a metallic mortar and pestle; 500 g of the resultant powder was then extracted using 1000 ml of diethyl ether (Fisher Scientific, Jersey, New Jersey, U.S.A.) for five days at 25°C. The extract was decanted and filtered using Whatman No.1 filter paper, followed by solvent removal using simple distillation at 36°C. The mark left on the filter paper after the filtration process was then extracted using 1000 ml of ethanol (PVT, Mumbai, India) at 25°C. The resulting extract was then decanted and filtered using Whatman No.1 filter paper followed by solvent removal using rotary evaporator in vacuo. The crude extract (41.5 g; 8.3%) was obtained and used in the assay and for phytochemical tests. Phytochemical screening gave positive results for tannins and saponins.

The *C. collinum* extract (200 mg) was initially dissolved in 25 ml of ethanol 95% v/v (PVT, Mumbai, India) to make a stock solution of 8 mg/ml. This was then diluted with an appropriate volume of distilled water (final volume of 20 ml) to make the required test solutions in 250 ml plastic beakers. Therefore, the final concentrations ranged from 0.0125–0.200 mg/ml. The method of Olwa-Odyek *et al*[9], was then used for screening of larvicidal activity in all the experiments with slight modification —IV instar *Ae. aegypti* larvae. Laboratory-reared strain maintained in the Insectary Unit of the Entomology Department at the Uganda Virus Research Institute (UVRI), Entebbe, Uganda. The Neemazal F extract from *A. indica* A. Juss (Neem) from Meliaceae family against which the action of *C. collinun* was compared and obtained from the Entomology Department of Uganda Virus Research Institute (UVRI), Entebbe, Uganda. Ten IV instar larvae were transferred into the test crude extract solutions (20 ml) in 250 ml plastic beakers. The beaker that served as a negative control had 0.5 ml of 95% v/v ethanol (PVT, India).

Approximately 2% ground mice feed was sprinkled over the contents of each beaker to serve as feed. Another set of nine 250 ml beakers containing Neemazal F, an extract of *A. indica* A. Juss was used as positive control[8]. The concentrations used for the control group ranged from 0.028–0.448 mg/ml. A beaker containing 0.5 ml of 95% v/v ethanol (PVT, Mumbai, India) served as a negative control in this case. The larvae were incubated in the test crude extract and Neemazal F extract over a 24 h period of continuous exposure at 25°C and 70–80% relative humidity in the beakers at which larval mortality was assessed. The larvae were considered dead when they showed no signs of movement even after touching with a glass rod.

Three replicates of each extract and control were performed in order to ascertain the consistency of the results (Tables 1 and 2). The corrected percent mortalities were analyzed using Abbott's formula[10]. The mortality data were analyzed using Prism Version 3 from which lethal concentration (LC_{50}) values (24 h) and 95% confidence intervals (CI) were deter-

mined. The LC_{50} value of the test extract was compared with that of Neemazal F reflecting the potencies of the two; the one with a lower LC_{50} value being more potent of the two.

The LC_{50} of *C. collinum* Fresen [shown by effective concentration 50 (EC_{50})] on IV instar larvae was 0.051 mg/ml (95% CI of 0.041–0.063 mg/ml) (Table 1) compared to 0.078 mg/ml (95% CI of 0.063–0.097 mg/ml for *A. indica* A. Juss (Neemazal F) (Table 2).

Table 1. Larvicidal effects of ethanolic extracts of *C. collinum* on IV instar larvae of *Ae. aegypti* after a 24 h treatment at room temperature

Concentration of the extract (mg/ml)	No. of larvae dead/No. exposed	Mortality
Control	0/30	0
0.0125	3/30	10
0.025	6/30	20
0.050	12/30	40
0.100	30/30	100
0.200	30/30	100

Table 2. Larvicidal effects of Neemazal F after a 24 h of treatment at 25°C

Concentration of the extract (mg/ml)	No. of larvae dead/No. exposed	Mortality
Control	0/30	0
0.028	6/30	20
0.056	12/30	40
0.112	15/30	50
0.168	24/30	80
0.224	27/30	90
0.280	30/30	100
0.336	30/30	100
0.392	30/30	100
0.448	30/30	100

The crude extract of *C. collinun* was found to be active on the IV instar larvae of *Ae. aegypti.* The larvicidal activity varied with the concentration and exposure. The larvicidal activity of *C. collinun* (LC_{50} = 0.051 mg/ml) was comparable to that of *A. indica* A. Juss with LC_{50}= 0.078 mg/ml. Neemazal F, an extract of *A. indica* A. Juss (was included in this study for comparison purposes). The LC_{50}= 0.078 mg/ml value of *A. indica* A. Juss in the present study supports earlier reported LC_{50} value regarding *A. indica* A. Juss alcohol extract on IV instar larvae of *Ae. aegypti* (LC_{50} = 0.023–0.083 mg/ml)[11]. The exact active principle in *C. collinum* responsible for the larvicidal effect has not yet been identified, but the *Combretum* sp of South Africa have been reported to contain sufficient amount of tetranortriterpenoids[7]. The observed mosquito larvicidal effects could possibly be due to these compounds[11].

Now-a-days, the major strategies for mosquito control are insecticides, mosquito nets, mineral oil larvicides, and mosquito repellents[5]. Unfortunately, most of these strategies are not sustainable for long-term use particularly in developing countries because of their prohibitive costs[5]. Mosquito nets are not effective against *Ae. aegypti* mosquitoes because of their aggressive day-time feeding habits[3].

Moreover, there is widespread resistance to available conventional insecticides[5]; even conventional aerial spraying with adulticides or sequential fogging gives no prolonged control of the target mosquito population[12]. A potential explanation for this phenomenon is that the surviving gravid females that were less affected by space spraying will continue their ovipositing and are joined by newly emerged mosquitoes from unaffected breeding sites[12]. According to Chung *et al*[12], routine use of chemical adulticide for outdoor purposes not only needs to be used in combination with spraying for indoor control, but also with appli-

cation of mineral oil to kill the larvae. Unfortunately, mineral oil is an environmental pollutant and hence not environment-friendly. To achieve both, an effective and a sustainable vector control strategy, is necessary to come up with an affordable, effective, safe and environment-friendly solution. The most promising strategy meeting these requirements lies in botanical insecticides hence much interest is now focused on plant extracts[11]. Therefore, we investigated the larvicidal activity of *C. collinum* Fresen shoot bark-ethanol extract by exposing the IV instar larvae to it and observing the larval mortalities.

In conclusion, we found the crude extract of *C. collinun* effecttive against IV instar larvae of *Ae. aegypti* and its larvicidal activity is located in the shoot bark which makes it a suitable candidate for the development of new larvicides, since its exploitation would not endanger the plant. Moreover, it may be potentially cheap to produce because its solubility in ethanol means that it has the potential to dissolve in water. Additional biological assays are planned to: (i) carry out toxicological tests on laboratory mice and rats to determine its safety; and (ii) determine exactly the constituent responsible for the larvicidal activity through bioassay directed fractionation.

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