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# Activity Guided Fractionation of Ethanol Extract of *Meriandra bengalensis* against *Anopheles* Mosquito Larvae

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author AK designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AFT and MTW managed the literature searches, performed the column chromatography and managed the experimental process. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** To prevent proliferation of mosquito borne diseases, to improve quality of life, mosquito control is essential. Research was aimed to find alternative eco-friendly and bio-degradable strategies in mosquito control at the larvae stage. Hence an attempt was made to study the larvicidal effect of the various column fractions of the ethanol extract of leaves of *Meriandra bengalensis* against late instar larva.
**Methods:** Dried and powdered leaves of *Meriandra bengalensis* were extracted with hexane and ethanol by continuous refluxing. Extracts were filtered and concentrated on rotary evaporator. Column chromatography was used to partially purify the ethanol extract using Alumina. Various polar fractions; 2%, 4%, 6%, 8% and 15% chloroform in petroleum ether and 10% methanol in

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chloroform were used to elute the active fractions. Larvicidal activity of various fractions at the 5 mg/ml and 2.5 mg/ml were recorded over 24 hour period. Larvae of *Anopheles gambie* were used for this study.

**Results:** All fractions showed very high larvicidal potential, Difference in larvicidal activity was observed with in the first 4 hours but later all fractions had a 100% mortality rate at both concentrations. At 5 mg/ml and 2.5mg/ml the strongest larvicidal activity was shown by the chloroform: pet ether (6%) which was equally active as the standard treatment. In the negative control all larvae were active and motile.

**Conclusion:** The findings of present study revealed that the column fractions of *Meriandra bengalensis* contain effective larvicidal agents. Further investigations are needed to elucidate this activity against a wide range of all stages of mosquito species and also the active ingredient(s) of the extract responsible for larvicidal activity should be identified.

*Keywords: Larvicidal; Meriandra bengalensis; column chromatography; Anopheles Gambie.*

## 1. INTRODUCTION

Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis (JE) causing millions of deaths every year [1]. Different approaches have been formulated to reduce the prevalence of malaria worldwide. The vector control arm of malaria prevalence reduction, using indoor residual spraying (IRS) of houses with synthetic insecticides, is challenged by the appearance of insecticide resistant vectors, and their adverse impact on the environment and ecological imbalance. Most of these chemical pesticides also have an adverse effect towards humans and animals and are not easily degradable spreading toxic effects. These insecticides may enter the food chain and thereby the liver, kidney & may result in irreversible damage of these organs. They may even result in mutation of genes and these changes become salient only after a few generations [2]. In Africa *Anopheles gambiae* s.s. mosquito is the vector that transmits malaria parasites. Every year 1.4-2.6 million people die out of the 300-500 million that are infected by malaria in Africa [3].

A vector is a living organism that can transmit infectious diseases between humans or from animals to humans. Many vectors ingest disease-causing micro-organisms during a blood meal from an infected host (human or animal) and their next blood meal they transfer the microorganisms to a new host. Mosquitoes are very efficient vectors [4]. Mosquito larvae (Fig. 1) can be found in a wide variety of habitats, ranging from temporary floodwater and snowmelt pools to more permanent water habitats like marshes, swamps, lagoons, and ponds; stagnant waters; and natural and artificial containers. Shallow water is ideal for larval survival because there is less turbulence and wave action. Upper water movement interferes with the surface feeding of some mosquito species, and in most species, it hinders the larvae and pupae from obtaining oxygen at the air-water interface. A deep-water environment prevents bottom-feeding larvae from reaching food that has accumulated at the lower levels of the water column. Larvae can grow in fresh as well as saline water with varying degrees of organic content [5].

**Fig. 1. Larvae of mosquito**

Mosquitoes are notorious pests of humans and other animals. Bites from mosquitoes can cause intense itching due to an immunological reaction to mosquito saliva [5]. The most common mode of transmission of malaria is through the bite of an infected female *Anopheles* mosquito. When a female *Anopheles* mosquito bites an infected person, some of the malaria parasites in the blood will be sucked into the mosquito. The malaria parasites multiply and develop in the mosquito. After 10-14 days they are mature and ready to be passed on to someone else. On her next meal the parasites are transferred to the

host's blood through the saliva of the mosquito. The plasmodium are then transported to the liver where they multiply and are released in to the general circulation. The malaria parasites can multiply 10 times every 2 days, destroying red blood cells on their release and infecting new cells throughout the body [6].

### 1.1 Strategies of Malaria Control in Eritrea

Integrated Vector Management (IVM), indoor residual spraying (IRS), Insecticide Treated Nets (ITNs), larvicidal, and environmental management strategies of the malaria control were used in Eritrea. IVM and IRS guidelines were developed in 2010 in order to guide implementation of IVM activities. IRS was implemented in targeted villages of Debub and Gash Barka zones, Eritrea, the coverage of which, according to MIS 2012, was 49.4% and 31.5% respectively. There has been active participation of communities in vector control activities as a result of intensive health promotional campaigns and provision of necessary materials [7].

*Meriandra bengalensis*, known as "nhba", is an annual herbaceous and aromatic plant belonging to the Lamiaceae family. A much branched erect shrub, 3-6 ft. high, with densely pubescent branches. Leaves petioled, blong-lanceolate, 2-3 inch. long, rugose, minutely crenate, with a scent like those of *Salvia officinalis*, but stronger. Traditionally it is used as an antiseptic, astringent, & for its antirheumatic, and carminative properties [8]. Essential oil of *Meriandra bengalensis* leaves from Yemen showed camphor (43.6%), 1,8-cineole (10.7%), borneol (3.4%), caryophyllene oxide (5.8%) and a-eudesmol (5.8%) as the major constituents [8].

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The mature leaves of *Meriandra bengalensis (native: Nhba)* were collected from Betgergish, Asmara and were authenticated by Dr. Ghebrehiwet Medhanie, botanist, Eritrean Institute of Technology (EIT), Mai Nefhi, Eritrea.

### 2.2 Preparation of Extracts

The mature plant leaves were washed with running tap water and shade dried for 7-14 days at room temperature. The dried leaves were powdered mechanically using commercial electrical stainless steel blender. Powdered leaves were sequentially extracted by soxhlet using n-hexane and ethanol, for 6 hrs each. The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The extract was concentrated under reduced pressure at 45℃ and the residue obtained was stored at 4℃.

### 2.3 Column Chromatography

Column chromatographic technique was used to partially purify the ethanol extract. Column (51x3 cm) was thoroughly washed with water & dried. Column was packed with Alumina (stationary phase) with petroleum ether as a mobile phase. Fine column packing was assured with constant taping of the column to remove the trapped air. Column was loaded with the free flowing slurry of the ethanol leaf extract. The petroleum ether (mobile phase) 1000 ml as the initial eluting solvent was used to start the column. Polarity of the mobile phase was increased by mixing with chloroform. Elution was based on gradient system and several fractions (10 ml each) were collected at the polarity of 2%, 4%, 6%, 8%, 15% & 25% chloroform in petroleum ether and finally eluted by mixing 10% methanol in chloroform to wash out the column. All the fraction of same polarity were combined together and concentrated at controlled temperature. All the dried samples were stored at 4℃ for larvicidal activity.

### 2.4 Collection and Identification of Mosquito Larvae

The *Anopheles gambie* mosquito larvae were collected from GezaKeren, AdiQuala subzone, Asmara, Eritrea. All the larvae were authenticated by Mr. Ghirmai Werede, Head of National Entomology laboratory, Mendefera, Eritrea.

### 2.5 Larvicidal Activity

The collected Mosquito larvae were kept in plastic trays containing water from their natural habitat. Larvae were divided into 8 groups consisting 10 larvae each and kept into a small transparent plastic cylindrical cups (10 cm in length & 6 cm in diameter) which contained 10 ml of water. Larvicidal activity was determined at the concentration of 5 mg/ml and 2.5 mg/ml. The desired concentration was added to the cups containing the mosquito larvae and kept under observation for 4 hrs.

Dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region. This was checked and confirmed at regular intervals. Percentage mortality was calculated for each group. A negative control (Organic solvent) and a positive control (Prallethrin) were also used in this study.

## 3. RESULTS

The activity of partially purified plant extracts is often attributed to the complex mixture of active compounds. In the preliminary screening, potential larvicidal activity of all the fractions was noted (Tables 1 and 2). The strongest larvicidal activity was shown by the 6% chlor/pet fraction, in which the mosquito larvae started to die within 15 minutes.

## 4. DISCUSSION

Identification of various plant extract that have larvicidal potential activities against mosquito can be of advantage in reducing the problem of resistance and concern for the environmental safety. Control of vectors is also a common way of disease control. Control of mosquito larvae can reduce the population of the insect which could be transformed into reducing the burden of the disease [9].

Column fractions of all the ethanol leaf extracts of *Meriandra bengalensis* exhibited larvicidal activity against *An. gambie* at 5 mg/ml and 2.5 mg/ml. There were no previous studies that compared larval mortalities induced by different concentrations of column chromatographic fractions of ethanol leaf extract of *Meriandra bengalensis* to the standard larvicidal(Prallethrin) on the late instar larvae of *An. gambie*.

In the present study, test concentrations of 5 mg/ml and 2.5 mg/ml of fractions of ethanol leaf extract of *Meriandra bengalensis had* similar effect as Prallethrin with all column fractions. The study also revealed all column chromatographic fractions of leaf extract of *Meriandra bengalensis to* have equal larvicidal activities after four hours with the 100% mortalities on the late instar larvae of *An. gambie in both concentrations*. The results were consistent after repeating the experiments using the same procedure. But there was no toxic effect seen with negative control which is strong evidence that the death of all larva were due to the active substance/s present in each column fractions. In the study, column chromatographic fractions of ethanol leaf extract of *Meriandra bengalensis was* shown to have ranged larvicidal potency/activity on the late instar larvae of *An. gambie..*, with the different fractions. At 5 mg/ml and 2.5 mg/ml the strongest larvicidal activity was shown by the 6% chloro

**Table 1. Mortality for 5 mg/ml**

| Concentration | Mean % Mortality ± SE | | | | |
|---|---|---|---|---|---|
| | 30 min | 1 hr | 2 hr | 3 hr | 4 hr |
| 2% Chlor/PetEther | 10 ± 0 | 20 ± 0 | 40 ± 0 | 70 ± 0 | 100 ± 0 |
| 4% Chlor /PetEther | 90 ± 0 | 90 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| 6% Chlor /PetEther | 100 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| 15% Chlor /PetEther | 90 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| 25% Chlor /PetEther | 90 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| 10% Meth/Chlor | 80 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| -ve Control | 0 | 0 | 0 | 0 | 0 |
| ±ve Control | 100 | 100 | 100 | 100 | 100 |

**Table 2. Mortality for 2.5 mg/ml**

| Concentration | Mean % Mortality ± SE | | | | |
|---|---|---|---|---|---|
| | 30 min | 1 hr | 2 hr | 3 hr | 4 hr |
| 2% Chlor/PetEther | 0 | 20 ± 0 | 40 ± 0 | 80 ± 0.5 | 100 ± 0 |
| 4% Chlor /PetEther | 40 ± 0 | 70 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| 6% Chlor /PetEther | 50 ± 0 | 90 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| 15% Chlor /PetEther | 40 ± 0 | 90 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| 25% Chlor /PetEther | 50 ± 0 | 80 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| 10% Meth/Chlor | 50 ± 0 | 90 ± 0 | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| -ve Control | 0 | 0 | 0 | 0 | 0 |
| ±ve Control | 100 | 100 | 100 | 100 | 100 |

in peth ether which has as equal activity as the standard treatment, Prallethrin, within a time frame of 30 minutes and 1 hour respectively.

The least toxicity on the test larvae was observed by column chromatographic fraction 2% chloro in peth ether in both concentrations. In all column fractions of *Meriandra bengalensis* the highest toxicity effect is at 5 mg/ml than 2.5 mg/ml concentration, indicating that concentration is directly proportional to potency/activity. Toxic activities of column chromatographic fraction at 4% and 8% chlro in petether were nearly equal to that of column chromatographic fraction 6% chloro in petether, this might be due to substances eluted at this range being same or similar in chemical properties.

There were toxic effects in all column fractions of ethanol leaf extract of *Meriandara bengalensis* showed larvicidal activity, with 6% chloro in pet ether having equal activity to Prallethrin at 5 mg/ml concentration, suggesting that the larvicidal activity of crude ethanol leaf extract of *Meriandara bengalensis* may not be due to the synergistic effects of its fractions.

## 5. CONCLUSIONS AND RECOMMENDA-TIONS

This study revealed the larvicidal effects of column chromatographic fractions of ethanol leaf extract of *Meriandra bengalensis* against the late instar larvae of *An. gambie*, the major vector of malaria in Eritrea. The following conclusions were made based on the findings of the study.

Column chromatographic fractions of ethanol leaf extract of *Meriandra bengalensis* showed better larvicidal activities, suggesting that the larvicidal activity of the Column chromatographic fractions of ethanol leaf extract is not due to the synergistic effects of the crude ethanol leaf extract of *Meriandra bengalensis.* Column chromatographic fractions of ethanol leaf extract had similar larvicidal activity.

Studies must be conducted to identify active chemical constituent/s for the mosquito larvicidal activities in the column chromatographic fractions of ethanol leaf extract of *Meriandra bengalensis* specially substance/s present in 6% chloroform in peth ether, the chemical entity/structure should be identified. In the future, wide scale studies need to focus on the larvicidal activities of crude ethanol leaf extract of *Meriandra bengalensis and* its column chromatographic fractions against *An. gambie* mosquitoes in Eritrea. Future field studies are also recommended so as to determine the residual activities of the ethanol leaf extract of *Meriandra bengalensis* and its effects on non-target organisms.

The plant *Meriandra bengalensis spps* should be cultivated by ministry of agriculture in collaboration with ministry of health in large scale. The activity of *Meriandra bengalensis* against all species of mosquito and other vectors should be tested. The parts of *Meriandra bengalensis* plants other than leaf should be also tested and its activities compared with leaf extract.

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## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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