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## Larvicidal activity of *Ocimum hadiense* against malaria vector anopheles mosquito

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### Abstract

The medicinal plant *Ocimum hadiense* is used against various illnesses. The plant was collected from Gomoro a sub zone of Mendefera and then washed with water and shed dried. The extraction involved sequential treatment of plant sample, using Soxhlet apparatus, with different solvents. The larvicidal activities of the crude extracts at a series of concentrations ranging from 50 to 400 mg/ml were tested against third instar larvae. The results suggest that the leaves and seed extract of *Ocimum hadiense* have been found effective in controlling mosquito larvae, which is why this plant is traditionally used in Eritrea and many other African countries. The formulation may prove to be an effective and eco-friendly larvicide. It has come to our attention that further research concerning the larvicidal properties of this plant should be done including toxicological studies, Purity- Activity Relationship and *in vivo and in vitro* antimalarial activities.

**Keywords:** *Ocimum hadiense*, larvicidal activity, anopheles mosquito, malaria

### Introduction

More than half the world is at risk of Vector-borne diseases, caused by pathogens and parasites in human populations. Every year more than one billion people are infected and more than one million people die from vector-borne diseases including malaria, dengue, schistosomiasis, leishmaniasis, Chagas disease, yellow fever, lymphatic filariasis and onchocerciasis [1]. Mosquitoes and ticks account for the majority of this transmissions, although some close relatives of mosquitoes also get involved, including sand flies and black flies [2]. The burden of these diseases is highest in tropical and subtropical areas, and they disproportionately affect the poorest populations [3], and therefore, several of them are listed at the WHO's list of 18 Neglected Tropical Diseases (NTDs). NTDs can be characterized because they have subsisted in the poorest and most marginalized societies, where the lack of adequate sanitation, and close contact with infectious vectors and reservoirs prevail [4]. Globally it is estimated 3.3 billion people are at risk of being infected with malaria and developing the disease, and 1.2 billion are at high risk (>1 in 1000 chance of getting malaria in a year) [5]. Ninety per cent of malaria cases in the world occur in Africa south of the Sahara in which Children under 5 years of age and pregnant women are the worst affected [6], that is caused by five species of parasites belonging to the genus Plasmodium. Four of these P. falciparum, P. vivax, P. malariae and P. ovale are human malaria species that are spread from one person to another via the bite of female mosquitoes of the genus Anopheles [7].

Mosquito control methods are available for preventing malaria and are being used widely; these take the form of insecticide-treated mosquito nets and indoor residual spraying. For these methods to be effective, high population coverage rates, exceeding 80%, are required. At such levels, they can reduce the risk for malarial disease by up to half, and, in low-transmission situations, they can have a similar or greater effect, including an impact on malaria infection rates. Thus, both of these preventive interventions can result in major reductions in malaria transmission and the related burden in all areas of the world, although there is no evidence that either one or the two in combination is effective enough to interrupt transmission in areas of high, stable transmission [8]. Progression towards malaria-free status is a continuous process [9].

### Material and Methods

#### Plant

*Ocimum hadiense* has fibrous roots with variably hairy, squarish, succulent stems without a tuberous base. Its leaves are 10-80 mm long, 8-55 mm wide cm Bright green to grey-green,

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ovate-triangular, ovate to rounded, succulent, opposite, surface densely glandular with velvety hairy and coarse from indentations on the upper surface of the complex network of veins. Individual flowers are 1-3 cm apart, sessile or with a 2-4 mm long pedicel, 8 - 13 mm wide, deep violet, purple, light blue or occasionally white and variably hairy. The fruits are 5-6 mm and the seeds are shiny, brown nutlets about 0.8 mm in diameter [10].

### Extraction

The plant material leaves and seeds of *Ocimum hadiense* were

washed with water and shade dried at 25-35 °C for 10-14 days. The dried plant matter was crushed and powdered for hot extraction. The extraction involved the sequential treatment of plant sample, using Soxhlet apparatus, with different solvents. The plant matter was extracted with three different solvents (table 1). The crude extractives were concentrated using Rota-vapor (BUCHI R-114) and were transferred to beakers and covered with glass covers and labeled. Samples were dried and stored in clean glass bottles for further use.

**Table 1:** Percentage yield of *Ocimum hadiense*

Plant	Solvent	Plant Powder	Yield amount	Percentage
	Petroleum ether	70g	3.118g	4.45%
<i>Ocimum hadiense</i>	Chloroform	70g	4.248g	6.061%
	Methanol	70g	4.02g	5.74%

### Mosquito larvae collection

The larvae of *Anopheles* mosquitoes were collected from a swampy area located in the southern region of Eritrea and the bioassay was done in Malaria Entomology Laboratory. The Larvae were kept in plastic trays containing tap water. They were maintained and all the experiments were carried out at a temperature of  $27 \pm 2$  °C.

### Larvicidal activity test

Larvae were taken in three batches of 10 individuals in 49 ml of water and 1.0 ml of the desired plant extract concentration. The control was set up with acetone. The numbers of dead larvae were counted after 6 hours of exposure and the percentage of mortality was reported from the average of three replicates (batches). Dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region. Moribund larvae are those

incapable of rising to the surface or not characteristic diving reaction when the water is disturbed.

### Result and Discussion

Exposure of *Ocimum hadiense* to three different solvents showed different larvicidal activity at different concentration and time of exposure that is methanol extract had the highest activity out of the other solvents extracts. Methanol extractives showed 100 % mortality after two hour of exposure at 400mg and 200mg and after six hour at 50mg concentration. And petroleum ether extractives showed 100% mortality after six hour of exposure at 400mg and 200mg concentrations. Whereas chloroform had the lowest activity that is after six hour of exposure it had 80%, 70% and 60% mortality at 400mg, 200mg and 50mg concentration respectively.

**Table 2:** Mortality Percentage of *Ocimum hadiense* on *Anopheles* larvae

Concentration	Mean Percentage $\pm$ Standard deviation											
	1hr			2hr			3hr			6hr		
	P.E	Ch	Me	P.E	Ch	Me	P.E	Ch	Me	P.E	Ch	Me
400mg	60 $\pm$ 0.81	40 $\pm$ 0.81	90 $\pm$ 0.57	80 $\pm$ 0	50 $\pm$ 0.81	100 $\pm$ 0	90 $\pm$ 0	60 $\pm$ 0.81	100 $\pm$ 0	100 $\pm$ 0	80 $\pm$ 0.57	100 $\pm$ 0
200mg	60 $\pm$ 0.81	40 $\pm$ 0.81	80 $\pm$ 0.81	80 $\pm$ 0.57	50 $\pm$ 0.81	100 $\pm$ 0	90 $\pm$ 0.57	60 $\pm$ 0.81	100 $\pm$ 0	100 $\pm$ 0	70 $\pm$ 0.81	100 $\pm$ 0
50mg	30 $\pm$ 0.81	20 $\pm$ 0.81	40 $\pm$ 0.57	50 $\pm$ 0.57	40 $\pm$ 0.57	80 $\pm$ 0.57	70 $\pm$ 0.81	50 $\pm$ 0.57	80 $\pm$ 0.57	80 $\pm$ 0.81	60 $\pm$ 0.57	100 $\pm$ 0

Key: P.E: Petroleum ether, Ch: Chloroform, Me: Methanol

### Phytochemical screening

A qualitative test analysis was carried out for the presence of different phytochemicals and the results are shown in table 4. The qualitative analysis done revealed the presence of

alkaloids, terpenoids, flavonoids, phytosterol, and phenol for *Ocimum hadiense*. The Presence of these components in this species shows that they may have other medicinal potential.

**Table 4:** Phytochemical analysis Results; (-) absent; (+) Present

No.	Name of Test	Test used	Observation	<i>Ocimum hadiense</i>
1	Test for Alkaloid	Wagner test	Red-brown Precipitate	(+)
2	Test for c.glycoside	Borntragers Test	Rose-pink color	(-)
3	Test for Saponin	Froth Test	1 cm foam	(-)
4	Test for tannin	Gelatin Test	White precipitate	(-)
5	Test for flavonoid	Alkaline Reagent Test	Yellow color to colorless	(+)
6	Test for terpenoid	Salkowaski test	Reddish brown at interface	(+)
7	Test for phytosterol	Salkowskis Test	Golden yellow color	(+)
8	Test for phenol	Ferric chloride Test	Bluish black color	(+)

Based on the preliminary screening results noted and presented in table 1 regarding percentage yield, three different crude solvent extracts of chloroform, methanol, and petroleum ether for *Ocimum hadiense* were subjected to

bioassay. Out of these, methanol extract of *O. hadiense* was found to possess the most effective larvicidal activity followed by petroleum ether extract and then chloroform extract based on percentage mortality against larvae of

*Anopheles mosquito.*

The varying results obtained in lethal concentration were probably due to the differences in the levels of toxicity among the insecticidal ingredients of the plant and the effect of plant extracts can vary significantly depending on plant species, plant part, age of plant part, solvent of extraction and mosquito species<sup>[11]</sup>.

**Conclusion**

The results suggest that the leaves and seed extracts of *Ocimum hadiense* have been found effective in killing mosquito larvae and this may be why this plant is traditionally used in Eritrea and other African countries. The formulation may prove to be an effective and eco-friendly larvicide, which could be used as an alternative for malaria prevention. *O. hadiense* was found to be effective and additional studies should be done even for other activities. In addition, it has come to our attention that further research concerning the larvicidal or antimalarial properties of this plant should be done.

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