

Larvicidal activity of *Cymbopogon citratus* (DC) Stapf. and *Croton macrostachyus* Del. against *Anopheles arabiensis* Patton, a potent malaria vector

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Abstract. – Methanol leaf extracts of two Ethiopian traditional medicinal plants viz., Lomisar [vernacular name (local native language, Amharic); *Cymbopogon citratus* (DC) Stapf. (Poaceae)] and Bisana [vernacular name (local native language, Amharic); *Croton macrostachyus* Del. (Euphorbiaceae)] were screened for larvicidal activity against late third instar larvae of *Anopheles arabiensis* Patton, a potent malaria vector in Ethiopia. The larval mortality was observed 24 h of post treatment. Both plant extracts demonstrated varying degrees of larvicidal activity against *Anopheles arabiensis*. *Cymbopogon citratus* extract has exhibited potent larvicidal activity than *Croton macrostachyus* at lower concentrations. The LC50 and LC90 values of *Cymbopogon citratus* were 74.02 and 158.20 ppm, respectively. From this data, a chi-square value 2.760 is significant at the $P < 0.05$ level. While, the LC50 and LC90 values of *Croton macrostachyus* were 89.25 and 224.98 ppm, respectively and the chi-square value 1.035 is significant at the $P < 0.05$ level. The present investigation establishes that these plant extracts could serve as potent mosquito larvicidal agents against *Anopheles arabiensis*. However, their mode of actions and larvicidal efficiency under the field conditions should be scrutinized and determined in the near future.

Key Words:

Plant extracts, Mosquito-larvicidal activity, *Anopheles arabiensis*, *Cymbopogon citratus*, *Croton macrostachyus*.

Introduction

Insect-transmitted diseases impose an enormous burden on the world's population in terms

of loss of life (millions of deaths per year) and morbidity. These diseases are also responsible for huge economic losses, both in terms of health-care costs and lost productivity, mostly in countries that can least afford them¹. Mosquito-borne diseases, such as filariasis, malaria, dengue, yellow fever, and Japanese encephalitis, contribute significantly to disease burden, death, poverty, and social debility in tropical countries².

Malaria, with 300-500 million new cases annually and approximately 2.5 million annual deaths, is one of the most devastating diseases affecting humans³. It kills more than 800,000 African children annually, mostly among the poorest that do not have access to adequate prevention and treatment⁴. Despite significant efforts to control malaria in Ethiopia since the 1950s, the disease remains one of the top public health problems in the country. An estimated 68% (50 million people) of the population lives in areas at risk of malaria⁵.

Because, in part, of rising drug resistance of the parasite, vector control is considered the most feasible way of controlling malaria in Africa today⁶. The extensive uses of synthetic organic insecticides during the last five decades have resulted in environmental hazards and also in the development of physiological resistance in major vector species. This has necessitated the need for search and development of environmentally safe, biodegradable and low cost indigenous methods for vector control⁷.

A number of plant families are known to produce alkaloids, phenolics and oils which have been used for insect control since a long time. They were called as insect killers and were used by Ro-

mans and Chinese⁸. Most of the problems of mosquito vector borne diseases occur in low-income tropical communities but these communities have the advantage of access to thousands of species of plants which may contain useful phytochemicals for control of both agriculturally and medically important insects⁹. Ethiopia is endowed with unique habitats that harbor many endemic species of plants. Of the 6500-7000 species of vascular plants in Ethiopia, 12% are endemic¹⁰.

The plant world comprises a rich storehouse of phytochemicals, which are widely used in the place of synthetic insecticides¹¹. Plants have been used since ancient times to repel/kill blood-sucking insects in the human history and even now, in many parts of the world people are practicing plants substances to repel/kill the mosquitoes and other blood-sucking insects¹². The phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents and ovipositional attractants¹³. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy to deal with them in this habitat. The use of conventional pesticides in the water sources, however, introduces many risks to people and/or the environment. Natural pesticides, especially those derived from plants, are more promising in this aspect¹⁴.

In recent epoch, around the globe phytochemicals has gained massive attention by various researchers because of their bio-degradable and eco-friendly values. Plants, *Cymbopogon (C.) citratus* and *Croton (Cr) macrostachyus* are well known for their medicinal and insects/mosquitoes repellent properties among the rural residents of Ethiopia. However, their mosquito-larvicidal activity is undetermined. Therefore, the purpose of the present investigation was to evaluate the larvicidal activity of these Ethiopian traditional medicinal plants viz., Lomisar [vernacular name (local native language, Amharic); *Cymbopogon citratus* (DC) Stapf. (Poaceae)] and Bisana [vernacular name (local native language, Amharic); *Croton macrostachyus* Del. (Euphorbiaceae)] against *Anopheles (An) arabiensis* Patton, the principal malaria vector in Ethiopia.

Materials and Methods

Selection of Plants Species

Plants, *C. citratus* and *Cr. macrostachyus* were selected from the secondary data i.e. some reports in the literature or some bio-ethnological

knowledge by the farmers, traditional healers and local residents. In Ethiopia, since the prehistoric eras the local rural residents have been using these plants for various medicinal and other purposes. The collected voucher specimens have been pressed, numbered, dried, identified and deposited in the Jimma University Regional Herbarium, Ethiopia.

Selection of Mosquito Species

Nearly all members of *Anopheles gambiae* complex, that are the potent vectors of malaria in Tropical Africa, have shown various degrees of resistance to DDT as well as to other organochlorine insecticides¹⁵. *An. arabiensis*, and *An. gambiae s.s.* are the most important vectors of human malaria in sub-Saharan Africa¹⁶. The available information about the present malaria vectors indicates that *An. arabiensis* is the major vector in Ethiopia^{17,18}. Thus, laboratory reared *An. arabiensis* was chosen for the evaluation of larvicidal activity. It was maintained at 27±2°C, 75-85% RH, under 14 L:10 D photoperiod cycles. The larvae were fed with dog biscuits and yeast at 3:1 ratio.

Plant Collection and Preparation of Plant Extracts

The leaves of *C. citratus* and *Cr. macrostachyus* were collected from outskirts of Jimma town, Oromiya Region, Ethiopia, and brought to the laboratory. The leaves were dried under shade at room temperature (29±1°C) for about 20 days. The completely dried leaves were powdered and sieved to get fine powder of leaf. The methanol-leaf extracts from the sieved fine leaf powder was obtained by using Soxhlet apparatus (ACMAS Technocracy [P] Ltd., Delhi, India). Two hundred and fifty grams of leaf powder was dissolved in 200 ml of methanol (as a solvent) and extracted in the Soxhlet apparatus for 8 h over a mantle heater at 55°C. The methanol extracts were concentrated using a vacuum evaporator (MEDICA Instrument Mfg. Co, Mumbai, India) at 45°C under low pressure. After complete evaporation of the solvents, the concentrated extracts were collected and stored in a refrigerator for later use.

Larvicidal Bioassay (WHO 1996)

Testing of the leaf extracts for larvicidal activity was done at different concentrations ranging from 10.0 to 60.0 mg/l by preparing the required stock solutions for the different concentrations

by following the standard procedure. The larvicidal activity of *C. citratus* and *Cr. macrostachyus* leaf extracts against late third instar larvae of *An. arabiensis* were determined by using WHO standard procedure¹⁹.

One percent stock solution of leaf extracts were made, from which other lower concentrations were prepared in acetone. The desired concentrations of test solution were achieved by adding 1 ml of an appropriate stock solution to 249 ml of tap water taken in a 500-ml beaker. Twenty-five numbers of late third instar larvae of *An. arabiensis* were exposed to various concentrations of *C. citratus* and *Cr. macrostachyus* leaf extracts. A pinch of larval food (125 mg) consisting of yeast powder and dog biscuit (1:1) were provided. The control experiments were also run parallel with each replicate. The larval mortality was calculated after 24 h of the exposure period. The experiments were conducted at 28±2°C and 70-80% relative humidity. The corrected percent of mortality was calculated by applying following Abbott's formula²⁰.

$$\text{Corrected mortality} = \frac{\text{Observed mortality (\%)} - \text{Control mortality (\%)}}{100 - \text{Control mortality (\%)}} \times 100$$

Statistical Analysis

The average larval mortality data were subjected to probit analysis for calculating LC₅₀, LC₉₀, and other statistics at 95% fiducial limits of upper confidence limit (UCL) and lower confidence limit (LCL), and chi-square values were calculated by using probit analysis²¹.

Results

The results of larvicidal activity of *C. citratus* and *Cr. macrostachyus* are presented in Table I, II and Figure 1. The methanol extracts obtained from the leaves of *C. citratus* and *C. macrostachyus* tested in different dilutions (60, 50, 40, 30, 20, and 10 ppm) have shown different degree of mortality against the malaria vector *An. arabiensis* Patton exposed to the test solution (Figure 1). The LC₅₀, LC₉₀, 95% confidence limits of LC₅₀, LC₉₀ and chi-square were also calculated. The LC₅₀ and LC₉₀ values of *C. citratus* were 74.02 and 158.20 ppm, respectively. From this data, a chi-square value 2.760 is significant

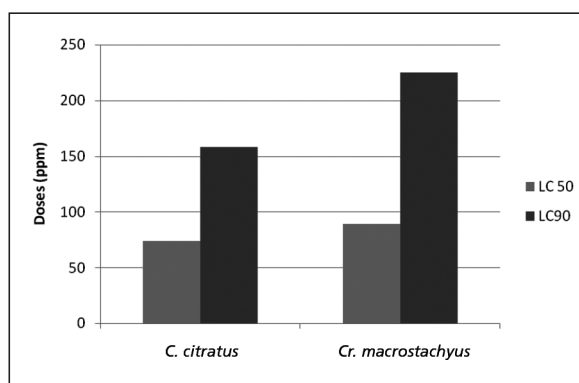


Figure 1. Larvicidal activity of *C. citratus* and *Cr. macrostachyus* on late third instar larvae of *Anopheles arabiensis*.

at the $P < 0.05$ level (Figure 1 & Table I). While, the LC₅₀ and LC₉₀ values of *Cr. macrostachyus* were 89.25 and 224.98 ppm, respectively and the chi-square value 1.035 is significant at the $P < 0.05$ level (Figure 1 & Table II). It was observed that at higher doses of *C. citratus*, the larvae turned immobile within an hour of exposure period.

Discussion

Our results showed that the leaf extracts of both *C. citratus* and *Cr. macrostachyus* are effective on *An. arabiensis*. However, *C. citratus* exhibited high level toxicity against *An. arabiensis* than *Cr. macrostachyus*. The results consistent with earlier report that volatile oils of three

Table I. Larvicidal activity of *C. citratus* on late third instar larvae of *Anopheles arabiensis*.

Bioefficacy	Value
LC50 (mg/l)	74.02
LC50 LCL	59.05
LC50 UCL	144.66
LC90 (mg/l)	158.20
LC90 LCL	98.96
LC90 UCL	773.70
Chi square (χ^2)	2.760*

UCL: Upper confidence limit; LCL: Lower confidence limit; LC: Lethal concentration.

Note: *Statistically significant at $p < 0.05$ level.

Table II. Larvicidal efficacy of *Cr. macrostachyus* on late third instar larvae of *Anopheles arabiensis*.

Bioefficacy	Value
LC50 (mg/l)	89.25
LC50 LCL	64.80
LC50 UCL	302.45
LC90 (mg/l)	224.98
LC90 LCL	117.58
LC90 UCL	3407.68
Chi square (χ^2)	1.035*

UCL: Upper confidence limit; LCL: Lower confidence limit; LC: Lethal concentration.

Note: *Statistically significant at $p < 0.05$ level.

Nigerian plants *Ocimum gratissimum* L., [Lamiaceae] *Cymbopogon citratus* (DC) Stapf [Poaceae] and *Ageratum conyzoides* L. [Asteraceae] were screened for larvicidal activity against larvae of *Aedes aegypti* L. The oil from *Ageratum conyzoides* was the most potent, giving 100% mortality at 120 ppm while oils from *C. citratus* and *Ocimum gratissimum* resulted in complete mortality at 200 and 300 ppm respectively²². In the present study, LC₅₀ value is slightly lower than a Nigerian study its might be possibly due to *An. arabiensis* higher susceptibility against *C. citratus* leaf extract than *Aedes aegypti*.

The screening of local medicinal plants for mosquito larvicidal activity may eventually lead to their use in natural product-based mosquito abatement practices. The essential oils obtained by hydrodistillation of dry leaves from *Cymbopogon citratus* (DC.) Stapf, *Ocimum canum* Sims, *Ocimum gratissimum* L. var "gratissimum" L. and *Thymus vulgaris* L. were analyzed for their larvicidal activity against fourth instar larvae of *Anopheles gambiae* Giles. These essential oils have remarkable larvicidal properties as they could induce 100% mortality in the larvae of *Anopheles gambiae* at the concentration of 100 ppm for *C. citratus*, 200 ppm with *Thymus vulgaris*, 350 ppm for *Ocimum gratissimum* and 400 ppm for *Ocimum canum*. The essential oil of *C. citratus* was found to be the most efficient, with respective values of: LC₅₀ = 18 ppm and LC₈₀ = 25 ppm²³.

The leaf extracts of *C. citratus* displayed a significant level of larvicidal activity against *An. arabiensis*. The result is comparable with earlier

findings. Sukumar et al.²⁴ reported that *C. citratus* causes significant growth inhibition and mortality in later developmental stages of *Aedes aegypti*. The final outcome of the present investigation with *C. citratus* quite comparable with an earlier report that in an effort to find effective and affordable ways to control mosquito, the larvicidal activities of essential oils from nine plants widely found in the Northeast of Brazil were analyzed by measurement of their LC₅₀. The results show that *Ocimum americanum* and *Ocimum gratissimum* have LC₅₀ of 67 ppm and 60 ppm respectively, compared to 63 ppm for *L. sidoides* and 69 ppm for *C. citratus*²⁵.

Even though, the toxicity level *Cr. macrostachyus* leaf extract lower than *C. citratus*, nevertheless it's also demonstrates significant level of mortality against *An. arabiensis*. The efficacy of *Cr. macrostachyus* leaf extracts regarded as moderate activity and the result is similar to earlier study. Out of the eight plant extracts tested against mosquito larvae, three possessed high larvicidal activity. These were *Randia nilotica*, *Gardenia lutea* and *Balanites aegyptiaca*. Four extracts, *Croton macrostachyus*, *Azadirachta indica*, *Aristolochia bracteata* and *Dioscorea dumetorum*, showed slight larvicidal activity, while the remaining one, *Vigna fragrans* did not possess any larvicidal activity²⁶.

The larvicidal activities of essential oils from *Croton* species widely found in northeastern Brazil were analyzed. The main components were methyleugenol and α -copaene for *Croton nepetaefolius* (LC₅₀ of 84 ppm); α -pinene and β -pinene for *Croton argyrophyloides* (LC₅₀ of 102 ppm); and α -pinene, β -phelandrene, and *trans*-caryophyllene for *Croton sonderianus* (LC₅₀ of 104 ppm). *Croton zenhtneri* exhibited higher larvicidal activity with LC₅₀ of 28 ppm, and the main active constituent was identified as anethole, a phenylpropanoid compound²⁷. Plants can provide safer alternatives for modern deadly poisonous synthetic chemicals. We are all just around the corner; to reinstate the chemical substances with plants derived one¹².

Vector-borne diseases are major causes of morbidity and mortality in many tropical and subtropical countries. Principally devastating nature of malaria in sub-Saharan Africa particularly in country like Ethiopia is indubitably intolerable. Indeed, source reduction is one of the key components in the malaria vector control programme since the target is exceptionally specific

unlike adult control. However, application of conventional insecticides into the mosquitoes breeding sites may lead to adverse side effects in the aquatic ecosystem. In this context, innovative vector control strategy like use of phytochemicals as alternative sources of insecticidal/larvicidal agents in the fight against the vector-borne diseases is become inevitable. The present study results evidently suggest that leaf extracts of Ethiopian traditional medicinal plants (*C. citratus* and *Cr. macrostachyus*) could serve as ideal potent larvicidal agents against *An. arabiensis*. However, their mode of actions and larvicidal efficiency under the field conditions should be scrutinized and determined in the near future.

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