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Bioactivity of essential oils of local plants against adult *Anopheles arabiensis* (Diptera: Culicidae) in Ethiopia*

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ABSTRACT

The adulticidal activities of essential oils of eleven plants namely *Chenopodium ambrosioides, Eucalyptus citriodora, Eucalyptus globules, Lippia adoensis, Mentha spicata, Nigella sativa, Ocimum lamiifolium, Ocimum suave, Piper nigrum, Schinus molle* and *Thymus vulgaris* were assessed against a laboratory colony of *Anopheles arabiensis* in Ethiopia. The Centers for Disease Control and Prevention (CDC) glass bottle bioassay was adopted to conduct bioefficacy tests. For each replicate, ten blood-unfed female *An. arabiensis* were exposed to different concentrations of essential oils coated in glass bottles, and mortality was recorded at intervals of 5 minutes for one hour to assess the mean percentage mortality and LC_{50} and LC_{90} values. The residual toxicity of six essential oils was also assessed by exposing adult *An. arabiensis* in nylon netting Barraud cages treated by oils. Of all the essential oils assessed for adulticidal activities, *O. suave* was found to be toxic at low concentration (LC_{50} = of 0.0014 ml% v/v; LC_{90} = 0.0027 ml% v/v). The next efficacious oil was that of *T. vulgaris* with LC_{50} and LC_{90} values of 0.0028 ml% v/v and 0.005 ml% v/v, respectively. The lowest activity was due to *S. molle, E. globulus* and *P. nigrum*. At a concentration of 0.05 ml% v/v, *O. suave* killed 100% of *An. arabiensis* within five minutes of exposure, while *P. nigrum* at the same duration caused similar rate of mortality at a concentration of 50 fold. Residual toxicity tests revealed *O. suave* to persist for 15 days, killing all mosquitoes in the first five days and 80% up to 10 days. The lowest residual activity was noted for *E. citriodora* which persisted only for 2 days. The essential oil *O. suave* acquired the highest level of toxicity at low concentration and within a short time. The efficacious nature of most plants has an implication for more screening of components of these plants with potential adulticides and develops for mosquito control.

Keywords: Adulticide; Essential Oils; *Anopheles arabiensis*; Residual Toxicity; Ethiopia

1. INTRODUCTION

Members of the *Anopheles gambiae* complex are the most important vectors of malaria in sub-Saharan Africa of which *Anopheles gambiae* s.s. and *Anopheles arabiensis* are the most widely distributed and most efficient vectors [1,2]. Vector control by the application of insecticides remains one of the most important strategies in the prevention and control of malaria. Indoor residual spraying (IRS) of insecticides and long lasting insecticidal treated nets (LLINs) are the two important tools utilized by a number of programmes. However, repeated application of insecticides is increasing the selection pressure for resistance in malaria vectors highlighting the need for new strategies for control [3] and plants products have received due attention as potential bioactive compounds against disease vectors.

Furthermore, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise a variety of components with different mode of actions [4]. Thus, the chance of developing resistance to plant products seems to be low [5]. Hitherto, the majority of research has focused on the

*Authors' contribution: FM involved in the study design and carried out the experiments, analyzed the data and interpreted the results; MT supervised the distillation of the essential oils, and involved in study design; MB supervised the bioassays and involved in the study design and interpretation of the results; TG supervised the bioassays and involved in the study design and interpretation of the results. FM and TG collected plants specimen. All authors read and approved the final manuscript.
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larvicidal and repellent activities of botanicals and only few have looked into their adulticidal effects [6,7]. Therefore, the aim of this study was to evaluate the adulticidal activities of some essential oils of local plants against adult *An. arabiensis* in Ethiopia.

2. MATERIALS AND METHODS

2.1. Collection of Plant Materials and Distillation of Essential Oils

A total of eleven plants were selected based on the traditional use as medicinal plants and information from the literature on their medicinal and/or insecticidal properties and were collected from different localities in Ethiopia. The plants included *Chenopodium ambrosioides* L., *Eucalyptus citriodora* Hook, *Eucalyptus globules* Labill., *Lippia adoensis* Hochst.ex Schau., *Mentha spicata* L., *Nigella sativa* L., *Ocimum lamiifolium* Hochst.ex Benth., *Ocimum suave* Willd., *Piper nigrum* L., *Schinus molle* L., and *Thymus vulgaris* L. Taxonomic identification of these plants was confirmed by professionals at the National Herbarium of Addis Ababa University. Prior to extraction of essential oils, moisture content of each sample was determined by using oven drying method at 100˚C. Essential oils were extracted from leaves and/or seeds of plants by hydro-distillation method using a Clevenger-type apparatus for 3 hours. The plant materials (leaves or seeds) were placed in a distillation flask and one liter of distilled water was added. The oil was separated from water and stored in airtight containers at 4˚C until later use.

2.2. Bioefficacy Tests to Determine Mortality

A population of *An. arabiensis* (Bishoftu strain: F_{51-61}) maintained at the Aklilu Lemma Institute of Pathobiology was served as test mosquito to determine mortality rates. Mortality bioassays were conducted in Pyrex glass bottles (capacity 250 ml) similar to the Centers for Disease Control and Prevention (CDC) glass bottle bioassay of insecticides [8]. Mortality was recorded at intervals of 5 minutes for an hour. The final mortality was recorded after 1-hour exposure.

Five milliliters (ml) of stock solution of each essential oil was prepared for four to five concentrations by diluting in acetone. For each test, fresh solutions at each concentration were prepared and the inner wall of a bottle was evenly coated with 1 ml stock solution from each concentration by shaking gently and rolling on a flat table. The contents were allowed to dry for at least two hours in a vertical position. Control bottles were treated with acetone alone. Ten 3 to 5 days old blood-unfed female *An. arabiensis* were introduced into a bottle and covered with a piece of cloth mesh by holding tightly with rubber band to prevent mosquitoes from escaping. Mosquitoes were provided with 10% sterile sugar solution soaked in cotton pads and placed on top of cover. The mosquitoes were considered dead if they were lying on their backs or sides at the bottom of bottle and unable to fly after gentle tapping of bottle. For each concentration, four replicates of treatment and two replicates of control were tested at room temperature.

2.3. Bioefficacy Tests to Determine Residual Toxicity

The residual bioefficacy of the essential oils against adult *An. arabiensis* was conducted in Barraud cages (15 × 15 × 15 cm) constructed from nylon netting. Only six essential oils were tested for their residual efficacy based on their relative toxicity determined from the one hour bioassay tests and availability of oils. The concentration that killed all test mosquitoes was selected and each cage was treated with 1% of oil diluted in acetone (v/v). Cages were allowed to dry in open air at room temperature for at least two hours and stored in polythene bags to maintain humidity from damp cotton pads. Ten female *An. arabiensis* (3 to 5 days old) were daily exposed to treated cages for 24 hours. As a food source, sugar-soaked cotton pads on filter paper were placed on top of each cage. Mortality was recorded after 24 hours of exposure. Four replicates of each treatment and two replicates of control (cages soaked in acetone only in equal volume) were conducted. Experimenting on each essential oil continued until the mean mortality was considered unsatisfactory.

2.4. Data Analysis

The mean of four replicates was taken to determine the mean percent mortality and time (in minutes) together with standard errors. Values of LC_{50} and LC_{90} and 95% confidence intervals were calculated by probit analysis and judged as significantly different between essential oils if the confidence intervals did not overlap [8,9]. The relative toxicity, defined as the toxicity of each essential oil relative to the least toxic essential oil was calculated as the LC_{50} value of least toxic essential oil divided by LC_{50} value of each essential oil as well as LC_{90} value of least toxic essential oil divided by LC_{90} value of each essential oil.

3. RESULTS

3.1. Adult Mortality

The toxicity of eleven plants essential oils against adult *An. arabiensis* is shown in **Table 1**. The most effective essential oil was *O. suave* which caused 100% mortality at a lowest concentration of 0.005 ml%, v/v. At the same concentration, only *T. vulgaris* caused 90% mortality.

Table 1. Toxicity of essential oils of different plants against adult *Anopheles arabiensis* after 1-hour exposure.

Plant	Conc.	% mortality (M ± SE)
O. suave	0.005	100
	0.0025	85 ± 0.28
	0.001	45 ± 0.28
	0.00075	15 ± 0.28
T. vulgaris	0.0075	100
	0.005	90 ± 0.0
	0.0025	40 ± 0.41
	0.001	15 ± 0.28
L. adoensis	0.075	100
	0.05	85 ± 0.28
	0.025	40 ± 0.0
	0.01	10 ± 0.0
M. spicata	0.075	100
	0.05	95 ± 0.28
	0.025	50 ± 0.28
	0.01	10 ± 0.0
C. ambrosioides	0.1	100
	0.075	75 ± 0.28
	0.05	25 ± 0.28
	0.025	10 ± 0.0
P. nigrum	1	100
	0.75	85 ± 0.28
	0.5	70 ± 0.41
	0.25	25 ± 0.28
E. citriodora	0.25	100
	0.1	90 ± 0.0
	0.075	75 ± 0.28
	0.05	10 ± 0.0
N. sativa	0.5	100
	0.1	90 ± 0.0
	0.25	55 ± 0.57
	0.075	15 ± 0.57
O. lamiifolium	0.5	100
	0.25	90 ± 0.0
	0.1	60 ± 0.41
	0.075	20 ± 0.41
E. globules	0.75	100
	0.5	90 ± 0.0
	0.25	65 ± 0.28
	0.1	15 ± 0.25
S. molle	0.75	100
	0.5	80 ± 0.41
	0.25	60 ± 0.41
	0.1	40 ± 0.41
	0.075	2 0 ± 0.41

M = means of four replicates, SE = standard error, *no mortality in control groups.

The rest plants were efficacious at higher concentrations ranging from the lowest 0.075 ml%, v/v (*L. adoensis* and *M. spicata*) to the highest 1 ml%, v/v (*P. nigrum*).

The time (in minutes) by which 100% of *An. arabiensis* dead varied among concentrations of the essential oils (**Table 2**). Thus *O. suave* killed all mosquitoes in five minutes with the smallest concentration (0.05 ml%, v/v) while *P. nigrum* required a higher concentration (2.5 ml%, v/v) to cause the same mortality rate in the same period of time. Similarly, at longer exposure times (15 - 60 min), the concentration of *O. suave* was much smaller than the rest of plants.

Table 2. Mean times (minutes) at which 100% mortality of *Anopheles arabiensis* induced by different concentrations of the essential oils.

Plant	Conc.	Time (M ± SE)
O. suave	0.05	5 ± 0.0
	0.025	15 ± 1.4
	0.01	30 ± 2.04
	0.005	60 ± 4.56
T. vulgaris	0.25	5 ± 0.0
	0.1	15 ± 2.04
	0.075	30 ± 2.88
	0.0075	60 ± 3.53
L. adoensis	0.5	5 ± 0.0
	0.25	15 ± 2.88
	0.1	25 ± 1.4
	0.075	60 ± 2.04
M. spicata	0.5	5 ± 0.0
	0.25	15 ± 3.5
	0.1	30 ± 3.5
	0.075	60 ± 2.04
C. ambrosioides	0.5	5 ± 0.0
	0.25	20 ± 2.04
	0.1	60 ± 2.88
P. nigrum	2.5	5 ± 0.0
	2	15 ± 2.04
	1.5	30 ± 2.04
	1	60 ± 2.88
E. citriodora	1	5 ± 0.0
	0.75	15 ± 2.04
	0.5	30 ± 2.04
	0.25	60 ± 4.10
N. sativa	1	5 ± 0.0
	0.75	25 ± 2.88
	0.5	60 ± 3.53
O. lamiifolium	1.5	5 ± 0.0
	1	15 ± 2.04
	0.75	30 ± 3.50
	0.5	60 ± 3.50
E. globulus	2	5 ± 0.0
	1.5	10 ± 2.04
	1	20 ± 2.04
	0.75	60 ± 2.04
S. molle	2	5 ± 0.0
	1.5	15 ± 2.04
	1	30 ± 2.88
	0.75	60 ± 4.56

M = means of four replicates, SE = standard error, *no mortality in control groups.

3.2. Values of LC_{50} and LC_{90}

LC_{50}, LC_{90} values and the relative toxicity of the essential oils is depicted in **Table 3**. The lowest LC_{50} value (0.0014 ml%, v/v) was that of *O. suave* while the largest (0.39%) was *P. nigrum*. All values show the essential oil

of *O. suave* to exhibit the highest toxicity to *An. Arabiensis.* The other plant with lower values of LC_{50} and LC_{90} but with greater relative toxicity was *T. vulgaris.* Although the values for *M. spicata* appear to be lower, its relative toxicity was much smaller than the two plants. The presence and absence of significant differences of LC_{50} and LC_{90} values among oils based on overlapping of confidence intervals is computed and shown in **Table 3**.

3.3. Residual Toxicities of Essential Oils

Six essential oils, *O. suave*, *S. molle*, *O. lamifolium*, *L. adoensis*, *E. citriodora* and *C. ambrosioides* were evaluated for their residual toxicity among which *O. suave* was observed to possess the longest residual activity, lasting for 15 days (**Figure 1**). In the first five days, mortality of *An. arabiensis* was 100% while from day six to ten, it was 80%. Its residual toxicity, however, was declined sharply and caused only 5% mortality at day15. The other plant with strong residual toxicity was *S. molle* which killed all mosquitoes for up to five days after which its residual went down. The lowest residual toxicity was that of *E. citriodora* essential oil which was effective for only day one.

Table 3. Values of LC_{50} and LC_{90} (ml%, v/v) of different plants essential oils against adult *Anopheles arabiensis*.

Plant	LC_{50} (95% CI)	LC_{90} (95% CI)	RT at LC_{50}	RT at LC_{90}
O. suave	0.0014 (0.0008 - 0.002)	0.0027 (0.002 - 0.006)	278.6	270.4
T. vulgaris	0.0028 (0.002 - 0.004)	0.005 (0.004 - 0.008)	139.3	146
M. spicata	0.027 (0.017 - 0.038)	0.047 (0.037 - 0.077)	14.4	15.5
L. adoensis	0.03 (0.021 - 0.04)	0.05 (0.042 - 0.082)	13	14.6
C. ambrosioides	0.06 (0.048 - 0.073)	0.09 (0.075 - 0.12)	6.5	8.1
E. citriodora	0.069 (0.055 - 0.08)	0.093 (0.081 - 0.127)	5.6	7.8
O. lamiifolium	0.12 (0.03 - 0.18)	0.24 (0.18 - 0.56)	3.3	3.0
N. sativa	0.13 (0.073 - 0.18)	0.24 (0.19 - 0.496)	3	3
S. molle	0.22 (0.081 - 0.34)	0.55 (0.4 - 0.96)	1.8	1.3
E. globulus	0.24 (0.11 - 0.34)	0.46 (0.35 - 0.79)	1.6	1.6
P. nigrum	0.39 (0.16 - 0.52)	0.73 (0.59 - 1.15)	1	1

CI = 95% confidence intervals (ml%, v/v); RT = relative toxicity at LC_{50} and LC_{90}.

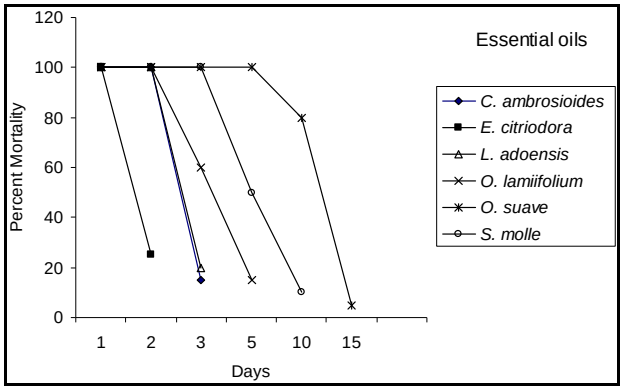

Figure 1. Residual toxicity of essential oils of different plants tested against *Anopheles arabiensis*.

4. DISCUSSION

The bioactivity results showed that the *O. suave* acquired the highest level of toxicity at low concentration. Nevertheless, the efficacious nature of the rest of the plants particularly *T. vulgaris*, *M. spicata* and *S. molle* could not be undermined. In terms of time, *O. suave* resulted in 100% mortality in five minutes at very low concentration compared to *O. lamiifolium*, *E. citriodora* and *E. globulus* respectively which required 30, 20 and 40 fold more concentration.

At very low LC_{50} values *O. suave* and *T. vulgaris* were greatly potent to *An. arabiensis.* The two plants were 278.6 and 139.3 times more toxic than that of *P. nigrum*, respectively. On the other hand, the essential oils of *C. ambrosioides* and *O. lamiifolium* were the most potent for larval stage of *An. arabiensis* and *Aedes aegypti* [10]. The essential oils of O. *suave* and *O. kilimandscharicum* have induced oviposition deterrence against gravid *An. gambiae* [11]. Moreover, fresh or smoked leaves *O. suave* and *O. kilimandscharicum* have been widely used as repellent for mosquitoes in north-eastern Tanzania [12]. Maharaj *et al.* [13] have conducted a cone bioassay of dichloromethane extracts of 381 plants against *An. arabiensis*, but only *Ptaeroxylon obliquum* and *Pittosporum viridiflorum* induced more than 50% mortality. Study on extract of *Tagetes minuta* against adult *Ae. Aegypti* and *An. stephensi* reported LC_{50} values of 0.15 and 0.16 ml%, respectively [14]. Choochote *et al.* [15] also noted adulticidal activity of ethanol extract of *Apium graveolens* with LC_{50} value of 6.6 gm/cm^2 against *Ae. aegypti*. In Thailand, the adulticidal effects of five essential oils were determined by topical application against *Ae. aegypti* and the LC_{50} values were reported between 5.44 to 8.52 µg/mg for laboratory strain and 5.54 to 8.83 µg/mg for field strain [6].

Concentration of essential oils is inversely related to mortality time. This association could be due to the increase of uptake of active ingredients by mosquitoes [16].

When mosquitoes were exposed to higher concentrations for 5 - 30 min, almost all showed signs of paralysis lying at the bottom of the bottle. But, the modes of actions of plant products could be different from the existing insecticides for vectors control [4].

The results revealed that the essential oils have residual activities against *An. arabiensis.* The residual activity of *O. suave* was highest (15 days) against *An. arabiensis* whereas that of the *E. citriodora* was the lowest (2 days). Essential oil of *Lantana camara* leaves on impregnated paper had residual activity against adult mosquitoes for 49 days [7]. But, further studies are required to identify the active components and to produce more effective formulations to elongate their residual activity. Screening more plant species is also needed to discover a large profile of plants that could be potentially useful in mosquito control programmes.

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