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# Larvicidal efficacy of Ethiopian ethnomedicinal plant *Juniperus procera* essential oil against Afrotropical malaria vector *Anopheles arabiensis* (Diptera: Culicidae)

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## PEER REVIEW

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## Comments

This is a valuable research work in which authors have demonstrated the mosquito-larvicidal activity of *J. procera* under the laboratory and field conditions. The activity was assessed based on WHO standard procedures. The essential oil of *J. procera* was found to be a promising larvicidal agent against insect vector of diseases. Details on Page S105

## ABSTRACT

**Objective:** To screen the essential oil of *Juniperus procera* (*J. procera*) (Cupressaceae) for larvicidal activity against late third instar larvae of *Anopheles arabiensis* (*An. arabiensis*) Patton, the principle malaria vector in Ethiopia.

**Methods:** The essential oil of *J. procera* was evaluated against the larvae of *An. arabiensis* under the laboratory and semi-field conditions by adopting the World Health Organization standard protocols. The larval mortality was observed for 24 h of post exposure.

**Results:** The essential oil of *J. procera* has demonstrated varying degrees of larvicidal activity against *An. arabiensis*. The LC<sub>50</sub> and LC<sub>90</sub> values of *J. procera* were 14.42 and 24.65 mg/L, respectively under the laboratory conditions, and from this data, a Chi-square value 6.662 was observed to be significant at the  $P=0.05$  level. However, under the semi-field conditions the LC<sub>50</sub> and LC<sub>90</sub> values of *J. procera* were 24.51 and 34.21 mg/L, respectively and a Chi-square value 4.615 was significant at the  $P=0.05$  level. The observations clearly showed that larval mortality rate is completely time and dose-dependent as compared with the control.

**Conclusions:** This investigation indicates that *J. procera* could serve as a potential larvicidal agent against insect vector of diseases, particularly *An. arabiensis*. However further studies are strongly recommended for the identification of the chemical constituents and the mode of action towards the rational design of alternative promising insecticidal agents in the near future.

## KEYWORDS

Essential oil, *Juniperus procera*, *Anopheles arabiensis*, Larvicidal activity, Ethiopia

## 1. Introduction

Mosquitoes serve as a vector for many dreadful diseases like malaria, filariasis, dengue fever, yellow fever and Japanese encephalitis. Malaria is considered to be a major public health issue in the resource-limited settings of Africa, Asia, and Latin America and beyond<sup>[1]</sup>. The World Health Organization Malaria Report<sup>[2]</sup> estimated that 3.3

billion people were at the risk of malaria in 2010, and of all geographical regions, populations living in sub-Saharan Africa have the highest risk of acquiring malaria; among 216 million episodes of malaria in 2010, approximately 81%, or 174 million cases, were observed to be from the African region. It was estimated that 91% of 655 000 of malaria deaths in 2010 were from Africa.

In Africa, malaria remains to be a disease of poverty and

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a cause of poverty<sup>[3]</sup>. Although malaria is both preventable and treatable, it has been a cause for severe maternal and childhood morbidity and mortality in Ethiopia<sup>[4]</sup> and kills a poor African child for every 60 seconds<sup>[2]</sup>. Besides health impact, it is also one of the major impediments to socio-economic development as the major transmission periods quite coincide with the peak agricultural season<sup>[5]</sup>.

It is well-known that the species within the *Anopheles gambiae* (*An. gambiae*) complex and the *Anopheles funestus* (*An. funestus*) group play a major role in the transmission of malaria in Africa<sup>[6,7]</sup>. The *An. gambiae* complex contains excellent and efficient vectors of malaria [*An. gambiae* s.s. and *Anopheles arabiensis* (*An. arabiensis*)], as well as minor vectors (*Anopheles merus*) and non-vectors (*Anopheles quadriannulatus* species A and B)<sup>[7]</sup>. In Ethiopia *An. arabiensis* serves as the major vector, while *An. funestus*, *Anopheles pharoensis* and *Anopheles nili* act as secondary vectors<sup>[8]</sup>.

The emergence of *Plasmodium falciparum* (*P. falciparum*) resistance strains, development of insecticide resistance, lack of reliable effective vaccine and unaffordable potential antimalarials<sup>[5]</sup> have imposed a serious negative impact on malaria control interventions. This causes vector control to be considered as a cornerstone to manage the vector populations by reducing/interrupting the cycle of disease transmission<sup>[9]</sup>. However, vector control programs are hampered by the continuous evolution of mosquito insecticide resistance and it is a potential threat to the global public health concern. The extensive application of conventional insecticides into the mosquitoes breeding sites may also lead to adverse side effects in the aquatic ecosystem<sup>[10]</sup>.

In this context, new alternative arsenals in terms of plant-based mosquitocidal agents with novel mode of action are urgently needed in the battle against vector-borne diseases particularly malaria. Since prehistoric period over thousand plant species have been employed to repel or kill various insects of public health and agriculture concern<sup>[11]</sup>. The identification and eventual use of indigenous plants for the mosquito larvae control may be much appreciated especially for developing countries as they are readily available and economical for use<sup>[12]</sup>. Currently, plant-based essential oils have received renewed attention as potent bioactive compounds against various species of mosquitoes<sup>[13]</sup>. Indeed, source reduction by targeting the larval stage of mosquitoes with potential larvicides is one of the most appropriate malaria vector controls strategy as the target is exceptionally specific unlike adult control<sup>[10,14]</sup>.

Essential oils are potentially suitable for mosquito larval control since they constitute a rich source of bioactive molecules that are effective and naturally biodegradable into non-toxic products<sup>[15–17]</sup>. Malaria control is highly dependent on the use of insecticide-based vector

control interventions. However, the evolution of insecticide resistance, the environmental and human health hazard, due to chemical warfare is a matter of grave concern. Therefore, there is an urgent need to screen and devise the resistance-proof, user and eco-friendly plant-based insecticides.

Even today in the resource-limited settings people are using numerous plant-based products as mosquitocidal agents despite their ethnic, cultural and socio-economic diversity. Globally, several researchers are keen to pursue with alternative vector control interventions by exploring our traditional knowledge into practice to devise potential plant-based insecticidal and repellent agents. It could pave the path for the reduction of overreliance on conventional insecticides and subsequently to minimize the environmental and human health hazards. In this context, the objective of the present investigation is to determine the larvicidal potential of *Juniperus procera* (*J. procera*) essential oil against Afrotropical malaria vector *An. arabiensis* under the laboratory and simulated field conditions.

## 2. Materials and methods

### 2.1. Selection of the plant

*J. procera* (Cupressaceae), was selected from the secondary data *i.e.* based on some reports in the literature or some bio-ethnological knowledge from the farmers, traditional healers and local residents<sup>[18]</sup>. In Ethiopia, the local rural residents have been using this indigenous plant for various curative and miscellaneous purposes. The collected voucher specimen has been pressed, numbered, dried, identified and deposited in the Jimma University Regional Herbarium, Ethiopia.

### 2.2. Taxonomy of *J. procera*

Kingdom	Plantae
Division	Pinophyta
Class	Pinopsida
Order	Pinales
Family	Cupressaceae
Genus	<i>Juniperus</i>
Species	<i>procera</i>

### 2.3. Description and traditional usage of *J. procera*

*J. procera* is commonly known in English as African Juniper or East African Juniper. It is a coniferous tree native to the mountains of eastern Africa from eastern Sudan south to Zimbabwe, and the southwest of the Arabian Peninsula. The stem is used as a tooth brush and leaves are used to treat or cure tonsillitis<sup>[19]</sup>. It has also been used to treat with

intestinal worm problems. The vapour from a leaf decoction has been inhaled several times a day for the treatment of flu like illness.

#### 2.4. Selection of mosquito species

Nearly all members of *An. gambiae* complex, that are the potent vectors of malaria in tropical Africa, have shown various degrees of resistance to commonly applied insecticides like dichlorodiphenyltrichloroethane (DDT) and pyrethroids<sup>[20]</sup>. *An. arabiensis* and *An. gambiae* s.s. are the most important vectors of human malaria in sub-Saharan Africa<sup>[7]</sup>, and *An. arabiensis* is the major malarial vector in Ethiopia. Thus, the Adama Malaria Research Centre, Ethiopia laboratory reared *An. arabiensis* was chosen for the evaluation of larvicidal activity. It was maintained at (27±2) °C, 75%–85% relative humidity, under 14 L:10 D photoperiod cycles. The larvae were fed with dog biscuits and yeast at 3:1 ratio.

#### 2.5. Plant collection and extraction

The leaves of *J. procera* was collected from the outskirts of Jimma town, Oromiya Region, Ethiopia, and brought to the laboratory. Taxonomic identification of the plants was performed by the botanists and taxonomists in the Department of Biology, College of Natural Science, Jimma University, Ethiopia. The leaves were washed with tap water, shade-dried at room temperature, mechanically ground by an electrical blender, and steam distilled at 100 °C for at least 6 h to obtain the essential oil. The solvent from the extract was removed using a rotary vacuum evaporator to collect the crude extract. Standard stock solutions were prepared at 1% by dissolving the residues in acetone. From this stock solution, different concentrations were prepared and these solutions were used to assess the larvicidal activity.

#### 2.6. Larvicidal bioassay under laboratory conditions

The *J. procera* essential oil was done at different concentrations ranging from 5 to 30 mg/L by preparing the required stock solutions for the different concentrations. The larvicidal activities of *J. procera* against *An. arabiensis* were determined by adopting WHO standard protocols<sup>[21]</sup>. One percent stock solution of essential oil was 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 *An. arabiensis* were exposed to various concentrations of *J. procera* essential oil. 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. Every bioassay was carried out in environmentally controlled

conditions [temperature ~ (25±2) °C; relative humidity ~ (80 ±10) %; 14 h light and 10 h dark cycle], and replicated four times with mosquitoes from different rearing batches. The percent of mortality was reported from the average of four replicates.

#### 2.7. Larvicidal bioassay under semi-field conditions

The *J. procera* larvicidal efficacy was also evaluated under the simulated field conditions by following the WHO standard protocols<sup>[21]</sup> and method of Mwaiko and Savaeli<sup>[22]</sup>. The artificial containers (plastic bowls) of 18 cm wide (diameter) by 7.5 cm depth of 1.5 L capacity were used for the bioassays in the semi-field and the containers were half-buried in the ground, and 249 mL of water from the natural breeding sites were added into each bowl. Each container was then treated with 1 mL of the stock solution of *J. procera* essential oil so that final volume was 250 mL each. The concentrations ranging from 15 to 40 mg/L were used for the tests in the semi-field conditions. Batch of 25 wild collected late third instar anopheline larvae were exposed into various test concentrations. Subsequently the containers were covered with nylon mosquito netting to prevent debris and other mosquitoes from egg laying. Four replicates were conducted for the treatments and two for the controls as described above. In both laboratory and field experiments, mortality and survival of larvae were determined after 24 h of exposure and the observations were also made on the behavior of larvae. The moribund and dead larvae in each concentration were combined in quadruplicate and expressed as percentage mortalities.

#### 2.8. Data analysis

It was important to obtain no less than three mortality counts of between 10% and 90%. In cases where the control mortality was between 5%–20%, the observed percent mortality (%M) was corrected by Abbott's formula<sup>[23]</sup>:

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

The average larval mortality data were subjected to probit analysis for calculating LC<sub>50</sub>, LC<sub>90</sub> and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit, and chi-square values were calculated using the SPSS12.0 (Statistical Package for Social Sciences: Chicago, IL, USA) software. Results with *P*<0.05 were considered to be statistically significant.

### 3. Results

The larvicidal activity of *J. procera* essential oil against late third instar larvae of *An. arabiensis* is presented in

Tables 1, Table 2, Figure 1, Figure 2 and Figure 3.

**Table 1**

Larvicidal activity of *J. procera* essential oil against *An. arabiensis* under laboratory conditions.

Concentrations (mg/L)	Mortality (%) (Mean±SD)	Larvicidal activity (95% CI, mg/L)	
		LC <sub>50</sub> (LCL–UCL)	LC <sub>90</sub> (LCL–UCL)
5	0		
10	28±4		
15	49.300±2.309		
20	64±4	14.42 (12.617–16.116)	24.65 (21.558– 30.253)
25	96±4		
30	100		
Control	0		
Chi-square( $\chi^2$ )	6.662		
R <sup>2</sup>	0.844		

Each value (mean±SD) represents mean value of three replicates.

LC<sub>50</sub>–Lethal concentration that kills 50% of the exposed larvae.

LC<sub>90</sub>–Lethal concentration that kills 90% of the exposed larvae.

UCL=Upper confidence limit.

LCL=Lower confidence limit.

**Table 2**

Larvicidal activity of *J. procera* essential oil against *An. arabiensis* under field conditions.

Concentrations (mg/L)	Mortality (%) (Mean±SD)	Larvicidal activity (95% CI, mg/L)	
		LC <sub>50</sub> (LCL–UCL)	LC <sub>90</sub> (LCL–UCL)
15	0		
20	33.300±2.309		
25	50.600±2.309		
30	70.600±2.309	24.51 (22.732–26.232)	34.21 (31.437– 38.801)
35	93.300±2.309		
40	100		
Control	0		
Chi-square( $\chi^2$ )	4.615		
R <sup>2</sup>	0.913		

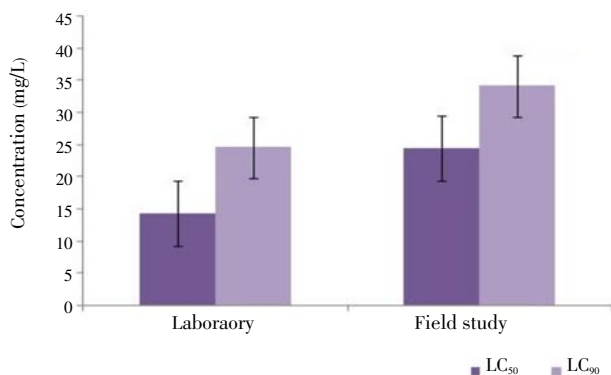
Each value (mean±SD) represents mean value of three replicates.

LC<sub>50</sub>–Lethal concentration that kills 50% of the exposed larvae.

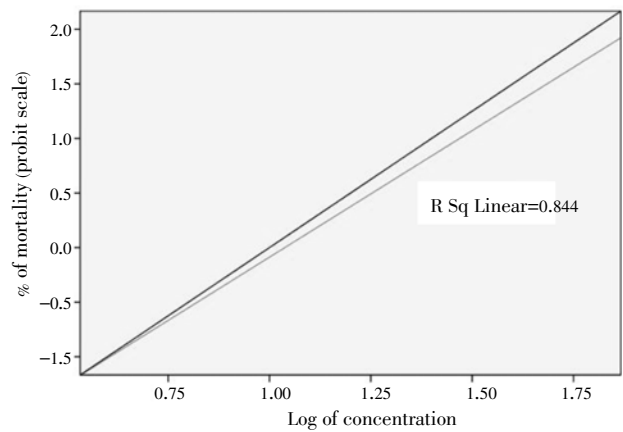
LC<sub>90</sub>–Lethal concentration that kills 90% of the exposed larvae.

UCL=Upper confidence limit.

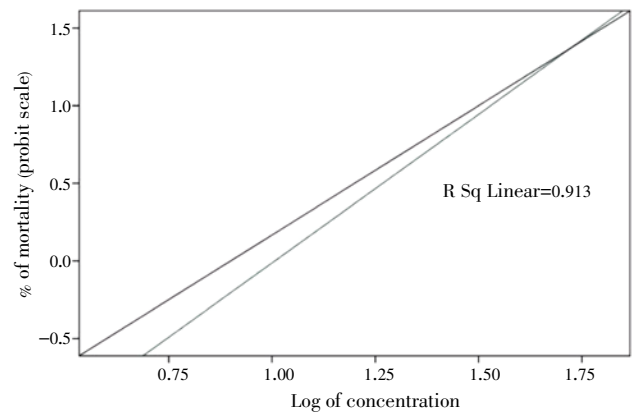
LCL=Lower confidence limit.



**Figure 1.** LC<sub>50</sub> and LC<sub>90</sub> values of the essential oil of *J. procera* against *An. arabiensis* under laboratory and field conditions.



**Figure 2.** Regression line of log concentration of *J. procera* essential oil against *An. arabiensis* under laboratory conditions.



**Figure 3.** Regression line of log concentration of *J. procera* essential oil against *An. arabiensis* under field conditions.

The obtained results established the potentiality of *J. procera* as a mosquito larvicidal agent. The LC<sub>50</sub> and LC<sub>90</sub> values were 14.42 and 24.65 mg/L, respectively under the laboratory conditions (Table 1, Figure 1 and Figure 2). In addition, the regression analysis indicates the mortality rate (*Y*) to be positively correlated with *J. procera* concentration (*X*) having a regression value (*R*) close to one in each case as shown in Figure 2 and Figure 3.

The larvicidal efficacy of *J. procera* essential oil against late third instar wild-collected anopheline larvae under the semi-field conditions is shown in Table 2, Figure 1 and Figure 3. It shows the highest larvicidal activity with LC<sub>50</sub>=24.518 and LC<sub>90</sub>=34.212 mg/L. The results clearly exhibit that the laboratory reared *An. arabiensis* larvae are more susceptible than the wild-collected anopheline larvae. The bioassay findings evidently suggest that the percent of mortality directly proportional to concentration and the rate of mortality were dose and time dependent (Figure 2 and Figure 3).

#### 4. Discussion

Indeed, malaria is one of the deadliest infectious diseases

in the resource–constrained settings. It is a disease of poverty inflicting a serious negative impact on human health and socio–economic development in the poorest countries of the world that cannot afford to succeed<sup>[3]</sup>. In Ethiopia, at the moment malaria control heavily relies on frontline vector control interventions like regular deployment of insecticide–treated nets and indoor residual spraying. However, their potentialities has been largely undermined due to the wide–spread emergence of insecticide resistance to the most commonly used insecticides like DDT and pyrethroids<sup>[9]</sup>, and due to resistance reported across sub–Saharan Africa and rest of the world too<sup>[24]</sup>.

Besides, there are no new classes of insecticides in the final stage of development<sup>[25]</sup>. In this perspective, the screening of locally available indigenous ethnomedicinal plants as mosquito larvicidal and adulticidal agents may eventually lead to their usage in plant–based mosquito abatement practices<sup>[10]</sup>. As per our best of knowledge and understanding so far there are no studies have been reported on the mosquito larvicidal activity of *J. procera*. Therefore, the present investigation was an attempt to explore on the promising novel mosquito larvicidal agent from an Ethiopian indigenous ethnomedicinal plant *J. procera*.

The results clearly suggest that the essential oil of *J. procera* was found to be the most effective one, against the late third instar larvae of *An. arabiensis* by providing 100% mortality at 30 mg/L with LC<sub>50</sub> and LC<sub>90</sub> values at 14.42 and 24.65 mg/L, respectively under laboratory conditions. The finding is in accordance with an earlier Ethiopian study reported by Massebo *et al.* that essential oils of other 11 local plants were evaluated for larvicidal activities against laboratory reared colonies of *An. arabiensis* and *Aedes aegypti* larvae<sup>[26]</sup>. The LC<sub>50</sub> values of the oils ranged from 17.5 to 85.9 mg/L against *An. arabiensis*. However, the LC<sub>50</sub> value of *Chenopodium ambrosioides* and *Ocimum lamiiifolium* essential oil were 17.5 and 20.9 mg/L, respectively against *An. arabiensis*. Similarly another Ethiopian study have indicated that the crude and column chromatographic fractions of methanol leaf extract of *Jatropha curcas* (*J. curcas*) were tested for their larvicidal activities against late third instar larvae of *An. arabiensis*. The crude methanol leaf extract of *J. curcas* have had similar larvicidal activity to 0.5 mg/L temephos (positive control) at test concentrations ranging from 125–1000 mg/L while column chromatographic fractions (F1 and F2) of the crude methanol leaf extract of *J. curcas* showed similar larvicidal activities to 0.5 mg/L temephos at 62.5 and 125 mg/L test concentrations<sup>[27]</sup>.

It is interesting to note that the larvicidal effect of *J. procera* essential oil against anopheline larvae under the semi–field conditions were LC<sub>50</sub>=24.51 and LC<sub>90</sub>=34.21 mg/L. Methanol leaf extracts of two Ethiopian traditional medicinal plants *Cymbopogon citratus* (*C. citratus*) and *Croton macrostachyus* (*C. macrostachyus*) were screened

for larvicidal activity against late third instar larvae of *An. arabiensis* Patton, a potent malaria vector in Ethiopia. *C. citratus* extract has exhibited potent larvicidal activity than *C. macrostachyus* at lower concentrations. The LC<sub>50</sub> and LC<sub>90</sub> values of *C. citratus* were 74.02 and 158.20 mg/L, respectively and the LC<sub>50</sub> and LC<sub>90</sub> values of *C. macrostachyus* were 89.25 and 224.98 mg/L, respectively<sup>[10]</sup>.

The mosquito larvicidal bioassay findings of the present study clearly demonstrated that the percent of mortality rate directly proportional to concentration and the mortality rate was highly dose and exposure time dependent. The results is in accordance with the findings of Elimam *et al.* which reported that the aqueous extracts from leaves of *Ricinus communis* against the *An. arabiensis* and the LC<sub>50</sub> values against the 2nd, 3rd and fourth instar larvae of *An. arabiensis* were 403.65, 445.66 and 498.88 mg/L, respectively<sup>[28]</sup>. Methanolic extracts of leaves and seeds from *Tribulus terrestris* was tested against 3rd instar larvae of *An. arabiensis* under laboratory condition. The seeds extract showed high insecticidal activity at all concentrations compared to the leaves extract and the LC<sub>50</sub> were 36.5 and 123.1 mg/L for seeds and leaves extract, respectively<sup>[29]</sup>. When comparing with the previous studies the present investigation results are quite noteworthy and remarkable even under the semi–field conditions. Therefore, the essential oil of *J. procera* could serve as a potential larvicidal agent under the field conditions as well.

The essential oils obtained by hydrodistillation of dry leaves from *C. citratus* (DC.) Stapf, *Ocimum canum* Sims, *Ocimum gratissimum* L. and *Thymus vulgaris* L. were analyzed for their larvicidal activity against the fourth instar larvae of *An. gambiae* Giles. These essential oils have remarkable larvicidal properties as they could induce 100% mortality in the larvae of *An. gambiae* at the concentration of 100 mg/L for *C. citratus*, 200 mg/L for *Thymus vulgaris*, 350 mg/L for *Ocimum gratissimum* and 400 mg/L for *Ocimum canum*. The essential oil of *C. citratus* was found to be the most efficient, with respective values of LC<sub>50</sub>=18 mg/L and LC<sub>80</sub>=25 mg/L<sup>[30]</sup>.

The present investigation results clearly demonstrated that the laboratory reared *An. arabiensis* larvae were found to be more susceptible to *J. procera* essential oil than the wild collected larvae of *Anopheline* population in terms of lethal concentration. The results are quite comparable with the previous studies, which have been conducted in Ethiopia. Three essential oils have already been tested to evaluate their larvicidal activity against third and fourth instars stage of wild–collected anopheline larvae in the simulated field conditions. The essential oil of *Ocimum lamiiifolium* showed highest larvicidal activity (LC<sub>50</sub>=34 mg/L and LC<sub>90</sub>=63.5 mg/L) followed by *Chenopodium ambrosioides* (LC<sub>50</sub>=47.3 mg/L and LC<sub>90</sub>=97.9 mg/L); the least activity was exhibited by *Piper nigrum* (LC<sub>50</sub>=110.6 mg/L; LC<sub>90</sub>=162.9 mg/L).



L). In all cases the wild-collected anopheline larvae have had higher  $LC_{50}$  and  $LC_{90}$  values of the essential oils than the laboratory reared *An. arabiensis* larvae[26]. It could be possibly explained that the wild anopheline larvae exposed to various chemical, biotic and non-biotic factors, might have contributed to the resistance development. In Ethiopia, several anopheline species such as *An. arabiensis*, *Anopheles paharoensis*, *An. funestus*, *Anopheles nili*, *Anopheles coustani*, *Anopheles marshallii* and *Anopheles demeilloni* had earlier been reported as malaria vectors, the former being the predominant one[31,32].

The present study results have shown clearly that the Ethiopian ethnomedicinal plant *J. procera* is highly efficacious for the control of mosquitoes under both laboratory and semi-field conditions. It was also evident that the percent of larval mortality rate was dosage dependent. The present results have been observed to concord with the previous study conducted in Sudan by Zarroug *et al.*, who tested some Sudanese plant extracts against *An. arabiensis* larvae and obtained significant mortality in the second and fourth instar larvae while using water extracts of *Balanites aegyptiaca*[33]. Very recently 381 crude plant extracts were investigated by Maharaj *et al.*[34] for their larvicidal effect on *An. arabiensis*, and plants were observed to exhibit promising results during screening and were selected for further phytochemical investigations. The results of the testing of the fractions generated identified one fraction of the plant *Toddalia asiatica* as being potent against the *An. arabiensis* larvae, among the rest of the tested plants.

Indeed the wide-spread *Plasmodium falciparum* chloroquine resistant strains have created severe set-back in malaria control. Though currently artemisinin-based combination therapy serves a pivotal role to combat malaria, they are mostly inaccessible and unaffordable in the resource-poor settings[35,36]. In this context, vector control is a mainstay in the malaria control programme of Ethiopia. It is well-known that the vector control interventions are highly depending upon the chemical insecticides such as DDT and pyrethroids. However, the extensive and injudicious use of synthetic insecticides in the vector control interventions has resulted in environmental and human-health hazards through persistence and accumulation of non-biodegradable toxic components in the ecosystem, development of insecticide resistance among mosquito species, biological magnification in the food chain and toxic effects on human health and non-target organisms[37,38].

In these perspectives, an ideal insecticide must meet or possess the intrinsic-qualities like low-cost, ready availability, easy-mode of application, pleasurable to use, undisruptive to environment, and safe for human and livestock. Nevertheless, the majority of the existing conventional insecticides are deficient to fulfill these decisive factors. In this prospect, plant-based insecticidal agents are relatively target specific, easily accessible, affordable, bio-degradable, and of user and environmental-

friendly nature. Currently, the evolution of multiple resistances among the insect disease vectors is an immense practical problem, challenging the existing vector-borne disease control success. In this context, over the past three decades there has been a renewed interest in designing potential plant-based resistance-proof insecticidal agents as plants possess a number of bio-active molecules and secondary metabolites resulting with complex mode of actions to tackle with the insecticide-resistance issue. Hence, further studies are required to be warranted for intensive screening and evaluation of mosquitocidal agents in terms of larvicidal, adulticidal, ovicidal, repellent and attractant from the traditional ethnomedicinal plant as a natural source of insecticides.

Despite several decades of control efforts still malaria remains to be the leading cause of morbidity and mortality in Ethiopia. At the moment, *Plasmodium falciparum* resistance strains and insecticide resistance, and unaffordable effective antimalarials have contributed to the resurgence and emergence of malaria incidence and malaria-related illness [36,39]. In this context, the *J. procera* plant, well-known by majority of the Ethiopians for its ethnomedicinal values, can be used as a phytotherapeutic agent to treat various ailments. In addition, it has been used as a traditional insect repellent to drive away mosquitoes and other blood-sucking insects by burning its leaves, barks and whole parts of plant in Ethiopia[40]. In the urban areas source reduction is one of the best options to combat mosquito-borne diseases like malaria, filariasis, yellow fever and dengue fever, as other vector control intervention like indoor residual spraying is quite unfeasible or impracticable.

Conclusively, the present investigation exhibited that *J. procera* could serve as a promising larvicidal agent against *An. arabiensis* under the laboratory as well as semi-field conditions with lesser  $LC_{50}$  doses. Since the candidate plant enjoys the widespread socio-cultural legitimacy among the majority of the Ethiopians, it enables us to implement as a sustainable alternative intervention in terms of an ideal mosquito larvicidal agent. Therefore, it could serve as a potential supplementary tool in the integrated malaria vector control strategy. However, further studies are strongly recommended for the isolation, identification, analysis, and larvicidal properties of *J. procera* bioactive ingredients and other secondary metabolites. In addition, the larvicidal mechanism and the toxicity towards the non-target beneficial organisms in the aquatic eco-system must also be scientifically validated. Besides, small-scale pilot studies have to be conducted to investigate its potential residual effects and associated health-risks in the near future.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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## Comments

### Background

Malaria is a leading public health issue in the resource-poor settings. It causes considerable mortality and threatens lives of half of the world population. Malaria is a disease of poverty and a cause of poverty. It is prevalent in the resource-poor settings of Africa, Asia and beyond. Therefore there is need of low-cost indigenous and eco-friendly plant-based insecticidal and repellent agents as vector control tool to address the emerging insecticide resistance issue.

### Research frontiers

The present research work depicts larvicidal activity of *J. procera* essential extract against *An. arabiensis* under the laboratory and semi-field conditions following WHO standard protocols.

### Related reports

Several studies reported the insecticidal activity of the plant-based products against various medically important insects particularly mosquitoes. However, only a limited number of studies carried out by the plants essential oils. This study has been performed with the essential oil of *J. procera*, an Ethiopian ethnomedicinal plant against a potent malaria vector in the Afro-tropical region, where malaria is more prevalent.

### Innovations and breakthroughs

There are studies demonstrated that *J. procera* can be used as a potent phytotherapeutic agent against various ailments. However, this is the first report on the mosquitocidal potentialities of *J. procera*.

### Applications

From the literature survey it has been found that *J. procera* is safe to humans. It has ethnomedicinal values against various ailments across Africa. This scientific study support

and suggest the use of this plant as a larvicidal agent, which could minimize the environmental pollution and adverse effect on human health.

### Peer review

This is a valuable research work in which authors have demonstrated the mosquito-larvicidal activity of *J. procera* under the laboratory and field conditions. The activity was assessed based on WHO standard procedures. The essential oil of *J. procera* was found to be a promising larvicidal agent against insect vector of diseases.

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