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Mosquitocidal efficacy of medicinal plant, *Nerium oleander* (Apocynaceae), leaf and flower extracts against malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae) larvae

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PEER REVIEW

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Comments

This article examined the mosquito larvicidal activity of *N. oleander* for its biomedical application. According to the results presented, the crude extracts of *N. oleander* had good lethal activity. In general, the paper is well structured; the methods are reproducible and the data are well presented. It is possible to develop a biodegradable insecticide from this plant.
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ABSTRACT

Objective: To assess the larvicidal efficacy of five different extracts of *Nerium oleander* L. (*N. oleander*) against malaria mosquitoes, *Anopheles stephensi* (*An. stephensi*), to combat disease.
Methods: The acetone, benzene, petroleum ether, chloroform, and aqueous extracts from leaves and flowers of *N. oleander* were subjected to distillation process in a Clevenger type glass apparatus model soxhlet. Larvicidal bioassays were performed to determine their LC_{50} and LC_{90} values, regression equation, and 95% confidence with lower and upper limits were calculated using probit analysis. Mortality effect was recorded after 24 h.
Results: The data indicated that there were significant differences in the lethality (LC_{50}, LC_{90}) values of *An. stephensi* mosquito larvae among most of the various extracts with their controls. The order of potency with five different concentrations of flower extracts was benzene>chloroform>acetone>petroleum>water. The order of potency with similarly five different concentrations of leaf extracts was chloroform>petroleum>benzene>water>acetone.
Conclusions: It was concluded that there was an overall lethal effect of *N. oleander* extracts against mosquito, *An. stephensi* larvae which could be manipulated to develop a safe and effective larvicide.

KEYWORDS

Mosquito, Larvae, *Nerium oleander*, Vector, *Anopheles*, Extract

1. Introduction

Human malaria is still one of the most important vector-borne health challenges particularly in the oriental parts of Iran, though it may be on the verge of elimination[1]. It is transmitted by mosquitoes which serve as infectious vectors of several other important potentially-fatal human parasitic diseases such as filariasis, dengue hemorrhagic fever and Japanese encephalitis[2]. These are major public health problems in most Asian and African countries because of their tropical or subtropical climates[3].

There are seven proven anopheline vectors of malaria

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including *Anopheles culicifacies*, *Anopheles stephensi* (Diptera: Culicidae) (*An. stephensi*), *Anopheles dthali, Anopheles fluviatilis, Anopheles sacharovi, Anopheles superpictus*, and *Anopheles pulcherrimus* in Iran[4]. The frequent use of insecticidal sprays and other chemical compounds to kill mosquitoes leads to a destabilization of the ecosystem and will affect the natural environment. Insecticide resistance is also increasingly becoming a challenge in many vector control activities. To evade these problems, a major emphasis has been made on the use of natural plant-based larvicides which could be a safe alternative to the synthetic insecticides[5]. Research is currently going on to discover environmentally-friendly products, such as the use of biological compounds, to eliminate the larval and adult mosquitoes. One such method is to use medicinal plants extracts against mosquito larvae in water bodies, since killing of the larvae is the weakest link in the life cycle of the malaria vector. It will terminate the natural life cycle and thus prevent further breeding of the mosquitoes which transmit malaria.

An. stephensi, an oriental malaria vector, is widely distributed in Indo-Persian region from India, Pakistan and Iran, to countries around the Persian Gulf. It occurs widely in the southern Iranian provinces of Kermanshah, Khuzestan, Fars, Kerman, Hormozgan, and Sistan-Baluchistan[6].

Nerium oleander L. (Gentianales: Apocynaceae) (*N. oleander*) is an evergreen small shrub of 2-5 m in height with a wide geographical and ecological distribution[7]. Oleanders are drought tolerant plants in the family Apocynaceae that is originated from the Mediterranean basin[8]. *N. oleander* displays white or pink terminal flower clusters which are visible on different branches[9]. All parts of the oleander plant are poisonous to humans and animals with repellent effect against certain insects[10-12]. But it is also proved to have various medicinal activities such as antibacterial[13-15], anti-inflammatory and antinociceptive[16,17], cytotoxic[18], antidiabetic[19], immunomodulatory[20], cardiotonic[21], and neuroprotective effects[22]. There are chemical compounds like oleandrin in the leaves of this plant.

Given these considerations, this plant is now being studied for its uses in medicine. In recent years, much effort has been focused on the exploration of bioactive chemical compounds from native plants for mosquito control in Iran. So, the purpose of the present study was to evaluate the larvicidal potential of *N. oleander* on malaria vector mosquito, *An. stephensi*, under laboratory conditions.

2. Materials and methods

2.1. Plant identification and preparation

In May 2011, the aerial parts including leaves and flowers of *N. oleander* (Figure 1) during blossoming stage were collected from the capital city of Ilam (54°13′N, 31°12′E, with an altitude of about 1427 m above sea level), Ilam province, on the western border of Iran with Iraq. The mean annual temperature is 23 °C and its mean annual precipitation rate is 620 mm. A voucher specimen was deposited in the herbarium of Eram botanical garden of Shiraz University, Shiraz, Iran. The fresh leaves and flowers were washed with distilled water, shade-dried at ambient temperature, finely powdered using an electrical stainless steel blender and stored in an air-tight container until further use. Morphological features of *N. oleander* were determined using Flora Iranica key[23] and a stereomicroscope.

Figure 1. Habitus of *N. oleander* plant showing the leaves and pink flowers on some branches.

2.2. Mosquito rearing

Early fourth-instar larvae of *An. stephensi* were used for bioassay tests. The laboratory bred *An. stephensi* (type strain, at Kazerun in Fars province) was reared in the insectarium at the department of medical entomology, Shiraz University of Medical Sciences (SUMS), and maintained at 27 °C with a photoperiod of 10 h light and 14 h dark at 75%-85% relative humidity. About 10% yeast suspension was used as food source ad libitum.

2.3. Isolation of plant extracts

The leaves and flowers were dried for 2 weeks in the shade at ambient temperature (27-35 °C days time). Air-dried plant material from the aerial parts of *N. oleander* was subjected to hydro-distillation for 3 h in a Clevenger type glass apparatus model Soxhlet with acetone, benzene, distilled-water, petroleum, or chloroform solvents. For each sample, 50 g of the plant materials (leaves or flowers) were separately extracted each time with 400 mL of solvent. After extraction, the resultant samples were dried on a rotary vacuum evaporator to delete water and kept in amber vial at 4 °C prior to the biological assays. The sample yielded 4% of solvent extracts and 18% of aqueous extract on a dry weight basis of 100 g.

2.4. Larvicidal bioassay

Ten different plant extracts were subjected to bioassay against 25 larvae in each replicate. The third and fourth stage larvae of *An. stephensi* (type strain) were kept in 500 mL glass beaker containing 249 mL of distilled water with 1.0 mL of different desired plant extracts derived from flowers or leaves with a concentration of 0.25, 0.5, 0.75, 1.0, 1.5, 1.75, 2.0, 2.5, 3.0 and 4.0 g/L. Four replicates for each concentration were set up. A control was also set up with 1.0 mL of acetone or aqueous solvent in 249 mL of distilled water. The control mortality was corrected by Abbott's formula[24] and the values for LC_{50}, LC_{90}, regression equation, and 95% confidence interval with fiducially lower and upper limits were calculated by using probit analysis[25].

3. Results

The current data indicated that there were significant differences in the LC_{50} and LC_{90} values of *An. stephensi* mosquito larvae among most of the various extracts of *N. oleander* with their controls ($P<0.05$). It was shown that there was a significant difference between the LC_{50} of larval mosquitoes using aqueous extract in relation to the other extracts ($P<0.05$).

Leaf extracts of *N. oleander* had the most and the least anti-larval effects with chloroform and acetone solvents, respectively. There was also a significant difference between the four different concentrations used ($P<0.05$), so that the LC_{50} of mosquito larvae at 2.5 g/L herbal extract was more effective than that at 0.5 g/L, indicating the higher concentration gave a bigger value for mortality (Table 1). The order of potency with similarly four different concentrations of leaf extracts was as follows: chloroform>petroleum>benzene>aqueous>acetone.

Table 1

Lethal concentration values for various leaf extracts of *N. oleander* against fourth stage larval mosquitoes of *An. stephensi*.

Extract	LC_{50} and LC_{90} (g/L)	Regression equation	95% Confidence limit		Slope±SE
			LCL:LC_{50} (LC_{90})	UCL:LC_{50} (LC_{90})	
Aqueous	1.865 (6.723)	$Y=3.016X+4.3768$	1.601(5.204)	2.124 (10.155)	2.3016±0.3000
Acetone	2.149 (6.949)	$Y=2.5145X+4.1644$	1.936 (5.166)	2.432 (11.633)	2.5145±0.3589
Chloroform	0.588 (1.747)	$Y=2.7099X+5.6243$	0.514 (1.440)	0.665 (2.289)	2.7099±0.2717
Petroleum	1.651 (3.477)	$Y=3.9644X+4.1358$	1.518 (2.966)	1.790 (4.477)	3.9644±0.5019
Benzene	1.772 (3.415)	$Y=4.4993X+3.8811$	1.647 (2.963)	1.910 (4.254)	4.4993±0.5380

LCL: lower confidence limit; UCL: upper confidence limit,

Flower extracts, on the other hand, using benzene and aqueous solvents gave the most and the least larvicidal effects, respectively. The order of potency with four different concentrations of flower extracts was as follows: benzene>chloroform>acetone>petroleum>water (Table 2).

Table 2

Lethal concentration values for various flower extracts of *N. oleander* against fourth stage larval mosquitoes of *An. stephensi*.

Extract	LC_{50} and LC_{90} (g/L)	Regression equation	95% Confidence limit		Slope±SE
			LCL:LC_{50} (LC_{90})	UCL:LC_{50} (LC_{90})	
Aqueous	2.221(5.6003)	$Y=3.1906X+3.8943$	2.0208 (4.5189)	2.4604 (7.9193)	3.1906±0.4081
Acetone	1.094 (5.933)	$Y=1.7450X+4.9310$	0.9010 (4.1950)	1.2970 (10.3870)	1.7454±0.2277
Chloroform	0.925 (3.211)	$Y=2.3706X+5.0802$	0.7890 (2.4680)	1.0540 (5.0200)	2.3706±0.3381
Petroleum	1.946 (3.986)	$Y=4.1261X+3.8036$	1.1370 (2.8110)	2.7450 (14.2680)	4.1261±0.6360
Benzene	0.551 (1.374)	$Y=3.2298X+5.8358$	0.4940 (1.1510)	0.6090 (1.7840)	3.2298±0.3623

LCL: lower confidence limit; UCL: upper confidence limit.

The LC_{50} and LC_{90} indices under all concentrations indicated that the benzene extract of flower and the chloroform extract of leaves had the most effective mortalities on larval mosquitoes. Benzene extracts were more effective in killing of mosquito larvae than the other extracts (Figure 2). The aqueous flower extract caused about 50% less mortality on *An. stephensi* larvae than the aqueous leaf extract.

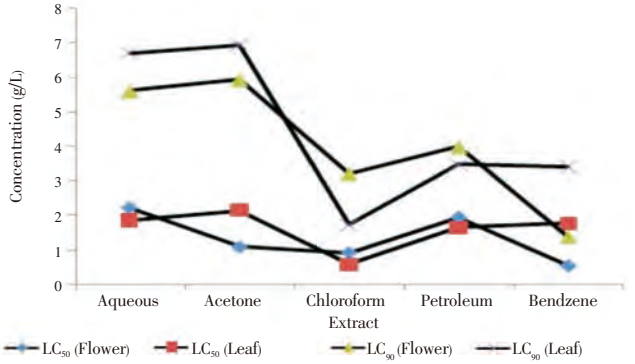

Figure 2. The relative lethality of different extracts from flowers and leaves of *N. oleander* plant on *An. stephensi* larvae.

4. Discussion

Arthropods transmit various diverse pathogenic agents with immense public health importance in Iran[26-29]. It was found that there was an overall lethal effect of *N. oleander* extracts against larval mosquitoes, *An. stephensi*, in Iran. Compared to the other extracts, the aqueous extract clearly revealed a weaker lethal activity against larval mosquitoes. The chloroform and benzene extracts exhibited marked lethal activity against these premature insects. Even though the number of reports on aromatic plants used in different countries for their larval mosquitocidal activities continues to increase, to the best of our knowledge, no studies have been published on the lethal activity of *N. oleander* against the malaria vector mosquito larvae, *An. stephensi*, in Iran.

Our research focused solely on LC_{50} and LC_{90} activity with instant effectiveness after the exposure, of which the highest was within 1 h of exposure to petroleum extract. Effectiveness is generally likely to be the most real factor in the search for noble herbal compounds. Apart from the direct impact of these compounds on lethality of mosquito

larvae, secondary impacts may culminate in reduced fecundity and eggs delivery[30].

The *Nerium* plant is native to Iran. It is thus conceivable to search for native means of combating larval mosquitoes. Some recent studies have addressed the phytochemical composition of *N. oleander* leaves having larvicidal effect against a few vector mosquitoes[8,31]. The various extracts of *N. oleander* plant exhibited high larvicidal activity against different stages particularly the fourth larval stage of *An. stephensi* mosquitoes, which is corroborated by other studies carried out elsewhere[12,32,33]. This plant appears to have active ingredients which could kill the mosquito larvae effectively. This fact may be used as a solution for the long lasting problem with mosquitoes in the developing countries without incurring any environmental damage[31]. This idea has been put forward by global agencies long ago[34].

It was thus concluded that there was a significant lethal effect of *N. oleander* leaf extracts containing chemicals like oleandrin against larval mosquitoes, *An. stephensi*, in southern parts of Iran.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Human malaria caused by the protozoan parasites, *Plasmodium*, and transmitted by the infectious bites of female mosquitoes, *Anopheles*, remains a formidable vector-borne health challenge particularly in the oriental parts of Iran. The potential use of medicinal plant extracts on mosquito larvae in aquatic habitats implicates a safe, effective and biodegradable alternative to the synthetic insecticides. The present study examined the larvicidal activity of *N. oleander* plant on malaria vector, *An. stephensi*.

Research frontiers

This research is conducted to evaluate the larvicidal activity of *N. oleander* plant. Bioactive chemical compounds in the leaf and flower of this plant are effective against specific target insects and are decomposable.

Related reports

Many papers have reported the efficacy of plant extracts against mosquito larvae such as Raveen *et al*. (2014), Roni *et al*. (2013), Kumar *et al*. (2012) and Sedaghat *et al*. (2011) who reported on the mosquitocidal efficacy of plant extracts against different larval species.

Innovations & breakthroughs

This research showed that the benzene extract of flowers and the chloroform extract of leaves of *N. oleander* plant had the most effective mortalities on larval mosquitoes. This finding is highly important in the field of pharmacology for the production of novel drugs to control the vectors of malaria.

Applications

It is valuable to find the use of commonly distributed plant (*N. oleander*) throughout the Southwest Asia, including Iran, for extraction of novel bioactive compounds with unique significance and medical application. Thus, it has been shown here that the benzene extract of flowers and the chloroform extract of leaves of *N. oleander* are pharmacologically important.

Peer review

This article examined the mosquito larvicidal activity of *N. oleander* for its biomedical application. According to the results presented, the crude extracts of *N. oleander* had good lethal activity. In general, the paper is well structured; the methods are reproducible and the data are well presented. It is possible to develop a biodegradable insecticide from this plant.

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