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Larvicidal activity of *Artemisia nilagirica* (Clarke) Pamp. and *Ocimum sanctum* Linn. A preliminary study

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Abstract

Objective: Aim of this work was to study the larvicidal activity of *Artemisia nilagirica* (Clarke) Pamp. and *Ocimum sanctum* Linn. against dengue, malaria, and filariasis transmitting mosquito larvae. Methods: The larvicidal activity of essential oil, chloroform, petroleum ether, and methanolic extracts of *A. nilagirica* and *O. sanctum* were tested against three different mosquito larvae *viz., Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*. Late third instar or early fourth instar larvae were selected for the screening. These extracts and essential oil were used for determining the larvicidal activity by using WHO method for evaluation of new larvicidal agent. Results: The result suggests larvicidal activity by essential oil, petroleum ether and chloroform extract of both the plants against all three selected mosquito species, while methanolic extract was found to be inactive up to 300 ppm. Among the essential oil and extracts of *A. nilagirica* and *O. sanctum*, essential oil of *O. sanctum* was found to be the most potent larvicide against *C. quinquefasciatus* and *A. aegypti* larvae, while *A. nilagirica* essential oil was more potent larvicide against *A. stephensi* larvae. Conclusion: The results suggest the use of the plants in insect control as an alternative method for minimizing the noxious effects of some pesticide compounds on the environment. Thus the essential oil of *O. sanctum* and *A. nilagirica* may deliver promising, more selective and biodegradable larvicidal agents.

Key words: *Aedes aegypti, Anopheles stephensi, Artemisia nilagirica, Culex quinquefasciatus*, Larvicide, *Ocimum sanctum*.

1. Introduction

Mosquitoes are responsible for transmission of more diseases than any other group of arthropods[1]. Several mosquito species belonging to genera *Anopheles, Culex* and *Aedes* are vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue, dengue hemorrhagic fever, and yellow fever. Thus, one of the approaches for control of these mosquito-borne diseases is the interruption of disease transmission by killing or preventing mosquito to bites. Herbal products with proven potential as insecticides or repellents

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can play an important role in the interruption of the transmission of mosquito-borne diseases both at the individual and community level.

However the discovery, development and use of synthetic organic insecticidal chemicals with persistent residual action not only overshadowed the use of herbal products as insecticides of choice against mosquitoes but also became the major weapon for mosquito control. Since the discovery of DDT, mosquito control approach has been almost completely based on synthetic organic insecticides.

But the extensive use of synthetic organic insecticides during the last five decades has resulted in environmental hazards and also in the development of physiological resistance in most vector species [2]. This has necessitated the need for search and development of environmentally safe, biodegradable, low cost, indigenous methods for vector control, which can be used with minimum care by individual and communities in specific situations.

The plant *Artemisia nilagirica* (Clarke) Pamp. (Asteraceae) is described in Ayurveda and Siddha, as a potent drug against a variety of ailments [3]. The oil was found to be a good insecticide & the freshly extracted essential oil from air-dried leaves showed anti-bacterial and anti-fungal activities [4].

The plant is also employed to keep away fleas and other insects. *Ocimum sanctum* Linn. (Labiateae), popularly known as Tulsi and Holy basil is one of the sacred herbs for Hindus in Indian subcontinent. It has a versatile role to play in traditional Indian systems of medicine [5]. Essential oil of *O. sanctum* possesses potent anthelmentic, anti-microbial and mosquito repellent activity [6].

The aim of the present work was to investigate the larvicidal activity of essential oil and different extracts of *A. nilagirica* and *O. sanctum* against three different mosquito species of health concern, in search of a new larvicidal agent.

2. Materials and methods

2.1 Plants

Aerial parts of *A. nilagirica* and *O. sanctum* were collected from Ootacamund during flowering stage in the month of July-August and authenticated by Dr. Sheshu Babu, Botanist, Department of Botany, Government Science College Ootacamund, a voucher specimen is deposited at department of Pharmacognosy, J. S. S. College of Pharmacy Ooty. The plant material was dried under shade and powdered.

2.2 Extraction

A. nilagirica and *O. sanctum* plant were powdered (500 gm) and extracted in Soxhlet with petroleum ether (40-60°C) and then with chloroform and methanol (2 L, each) in succession. The extract was concentrated under reduced pressure to a semisolid mass. The essential oil of *A. nilagirica* & *O. sanctum* plant was obtained by hydrodistillation using Clavenger apparatus. These extracts and essential oil were used for determining the larvicidal activity against all three mosquito species.

2.3 Biological assay

Larvicidal activity was evaluated in accordance to World Health Organization (WHO) for the evaluation of new larvicidal agent [7]. The larvae of *A. aegypti A. stephensi*, and *C. quinquefasciatus* were obtained & reared from the neonates in National Institute of Communicable Diseases, Southern India Branch, Conoor, T.N. India, at 28 ± 2°C with a photoperiod of 12 h light and dark and 80 ± 10% RH. A Brewer's yeast powder mixed with an equal quantity (w/w) of ground dog biscuits is used in the laboratory as a food for larvae.

The late third or early fourth instar larvae were collected according to larval size and the degree

Table I
Larvicidal activity of *Artemisia nialgirica* against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* larvae

	***Anopheles stephensi*[a]**		***Aedes aegypti*[a]**		***Culex quinquefasciatus*[a]**	
Sample	**LC_{50} (ppm)[b] [UL,LL]**	**LC_{90} (ppm)[b] [UL,LL]**	**LC_{50} (ppm)[b] [UL,LL]**	**LC_{90} (ppm)[b] [UL,LL]**	**LC_{50} (ppm)[b] [UL,LL]**	**LC_{90} (ppm)[b] [UL,LL]**
Essential	30.54	58.56	55.06	73.36	62.34	93.87
Oil	[26.04, 40.44]	[50.54, 66.84]	[48.00, 61.56]	[66.65, 87.93]	[57.87, 77.55]	[84.35, 103.73]
Petroleum ether						
extract	50.35	94.99	88.83	145.56	98.93	163.84
	[43.01, 57.18]	[82.10, 117.09]	[78.70, 98.08]	[127.91, 180.55]	[82.70, 113.07]	[139.58, 222.18]
Chloroform extract	88.56	150.65	122.17	202.56	154.44	243.58
(Succ)	[80.36, 100.53]	[130.76, 186.75]	[113.46, 138.68]	[190.88, 233.56]	[144.98, 161.11]	[229.51, 263.88]

a Value of thrice in triplicates observations
b Values are mean ± (S.D.); [95% Confidence limits; UL. Upper limit; LL. Lower limit]

Table II
Larvicidal activity of *Ocimum sanctum* against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* larvae

	Anopheles stephensi[a]		*Aedes aegypti*[a]		*Culex quinquefasciatus*[a]	
Sample	**LC_{50} (ppm)[b] [UL,LL]**	**LC_{90} (ppm)[b] [UL,LL]**	**LC_{50} (ppm)[b] [UL,LL]**	**LC_{90} (ppm)[b] [UL,LL]**	**LC_{50} (ppm)[b] [UL,LL]**	**LC_{90} (ppm)[b] [UL,LL]**
Essential Oil	53.74	82.48	45.67	60.61	25.52	55.27
	[47.96, 59.49]	[72.43, 103.16]	[37.97, 52.35]	[52.78, 89.35]	[16.54, 34.17]	[39.93, 132.26]
Petroleum ether	87.58	167.45	79.58	175.31	36.94	160.36
extract	[79.62, 94.50]	[154.34, 189.54]	[72.72, 86.43]	[153.03, 197.58]	[30.99, 45.10]	[52.98, 79.36]
Chloroform extract	157.58	253.73	129.24	226.56	55.18	103.63
(Succ)	[140.62, 185.45]	[244.72, 283.53]	[121.00, 137.47]	[205.70, 247.41]	[40.62, 63.62]	[93.62, 120.39]

[a] Value of thrice in triplicate observations
[b] Values are mean ± (S.D.); [95% Confidence limits; UL. Upper limit; LL. Lower limit]

of chitinization of respiratory siphon [8]. Different concentrations of the extracts were prepared freshly in 1 ml of acetone for each experiment.

All experimental exposure was done in 500 ml glass beakers in triplicate. Twenty-five larvae were collected with a Pasteur pipette, placed on a filter paper for removal of excess of water and placed in 250 ml dechlorinated tap water containing various concentrations of the crude extract. Two controls in triplicate were set up, one with acetone (1 ml) and the other with distilled water (250 ml).

The beakers were covered with muslin cloth to avoid the entry of any foreign material. Sufficient controls were also kept for each extract and volatile oil. Mortality was recorded at the end of 24 h by counting dead and moribund larvae. Dead larvae were those, which could not be induced to move when they were probed with a needle in the siphon or the cervical region.

Moribund larvae were those incapable of rising to the surface (within reasonable time) or showing characteristic diving reaction when water was disturbed. After 24 h, crude mortality was recorded and corrected for control mortality by using Abott's formula.

2.4 Statistical analysis

LC_{50} and LC_{90} values and their 95% confidence limits were estimated by fitting a probit regression model to the observed relationship between percentage mortality of larvae and logarithmic concentration of the substance. Separate probit models were fitted for each species within each substance [9]. All analyses were carried out using the SPSS software, version 9.0.

3. Results and discussion

The larvicidal bioassay of *A. nilagirica* results suggest that among essential oil and extracts, essential oil exhibited highest activity against the *A. stephensi, A. aegypti,* and *C. quinquefasciatus* larvae and least larvicidal activity against *C. quinquefasciatus* larvae. The LC_{50} and LC_{90} values for the extract and essential oil of *A. nilagirica* were given in table I.

The essential oil of *O. sanctum* was found to be the most potent larvicide for all the three mosquito species in the following decreasing order, *C. quinquefasciatus, A. aegypti* and *A. anopheles* larvae. The results of susceptibility of larvae for the extract and essential oil of *O. sanctum* were given in table II.

The essential oil of *O. sanctum* was found to be more potent against *Culex* and *Aedes* larvae, while the essential oil of *A. nilagirica* was more potent against *Anopheles* larvae. The methanolic extract of both the species was found to be inactive up to 300 ppm for all the three mosquito species screened. Though many plants have been shown to possess larvicidal activity against mosquitoes, most of these reports are based on laboratory observations only. Products of some plants are effective at very high concentrations and thus may not be of much practical importance.

However, some of the plant products have shown promise for mosquito control even under field conditions. One of the most commonly studied plants for control of mosquitoes is *Azadirachta indica,* (Meliaceae) commonly known as neem in India. Neem oil and other commercial preparations of neem have been found as potential mosquito larvicide [10].

In conclusion the use of plants in insect control offers a safer alternative to synthetic chemicals and can be obtained by individuals and communities easily at a very low cost. Moreover, these results could be useful in the search for newer, more selective and biodegradable larvicidal natural compounds. Further investigations are currently underway to isolate the compounds responsible for larvicidal activity of these plants.

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