International Journal of Medicine and Pharmaceutical Research

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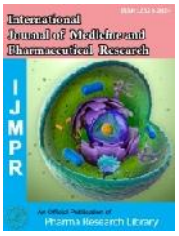

Research Article **Open Access**

The challenging larvicidal activity of *Ocimum sanctum*

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A B S T R A C T

Mosquitoes are the main vectors that are responsible to transmit various diseases, causing mortality and the development of resistance to chemical insecticides which ultimately results in rebounding vectorial capacity. These diseases could be prevented by using herbal plant extracts. In this study, the role of larvicidal activity of ethanolic extract leaf and stem of *Ocimum sanctum* against *Anopheles subpictus* Grassi and *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). It was concluded from the study that the ethanolic extract of leaves and stem of *Ocimum sanctum* could be used as an effective larvicidal agent as it is an ecofriendly method for the control and management of *Anopheles subpictus* and *Culex tritaeniorhynchus*.

Keywords: Larvicidal, eugenol, *Anopheles subpictus* and *Culex tritaeniorhynchus*

A R T I C L E I N F O

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Article History: Received 04 December 2016, **Accepted** 29 January 2017, **Available Online** 10 February 2017

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Manuscript ID: IJMPR3264

PAPER-QR CODE

Citation: Pragati Khare, *et al.* The challenging larvicidal activity of *Ocimum sanctum*. *Int. J. Med. Pharm. Res.*, 2017, 5(1): 01-04.

1. Introduction

Tulsi is one of the crucial symbols of tradition of Hindus. Its common name in English is Holy Basil and its botanical name is *Ocimum sanctum*, belonging to Lamiaceae family [1]. There are two types of tulsi, i.e. Krishna Tulsi (black) and Rama Tulsi (green). Tulsi means “incomparable one” in Sanskrit and is very religious to Hindus [2]. Tulsi has been used since long for its potent medicinal properties. *Ocimum sanctum* is traditionally used for treating cough, wound, bronchitis, liver diseases, cold, catarrhal fever, lumbago, hiccough, gastric disorders, genitourinary

disorders, skin diseases and psychosomatic stress disorders. *Ocimum sanctum* is gifted with aromatic, stomachic, carminative, demulcent, diaphoretic, diuretic, expectorant, vermifuge and febrifuge characteristics [1]. *Ocimum sanctum* was originated from Iran, Afghanistan and India. This plant is an annual spice plant [3]. *Ocimum sanctum* is erect, widely branched and aromatic herb, about 75 cms high. The main chemical constituents present in Tulsi are eugenol and ursolic acid [4]. It has been reported that *Ocimum sanctum* possesses chemical ingredients like eugenol, cardinene, phosphrous, cubenol, borneol, gallic acid, linoleic acid, linolenic acid, oleic acid, vallinin acid, palmitric acid, steric acid, iron, vallinin, vicenin, vitexin, orientin, circineol, Vitamin A and Vitamin C [2].

Chemical constituents in various parts of Tulsi

Seeds: Linoleic acid, linolenic acid, oleic acid, palmitric acid and stearic acid [5].

Leaves: Benzaldehyde, myrcene, borneol, bornyl acetate, camphor, caryophyllene oxide, camphene, cis-terpineol, cubenol, cardinene, d-limonene, eucalyptol, eugenol, farnesol, furaldehyde, germacrene, eicosane, heptanol, linalool, humulene, limonene, n-butylbenzoate, ocimene, oleic acid, sabinene, selinene, phytol, veridifloro, pinene, thujene, guaiene, gurjunene and methyl chavicol [6,7,8].

Figure 1: Tulsi Plant

Whole Plant: Vitamin C, Vitamin A, iron, calcium, chromium, copper, zinc and phosphorus [9].

Leaves/ Areal Parts:

Urosolic acid, aesculectin, aesculin, apgenin, vallinin acid, caffiec acid, chlorgenic acid, circineol, gallic acid, galuteolin, isorientin, isovitexin, luteolin, orientin, stigmsterol, vallinin, viceni, vitexin and molludistin [10].

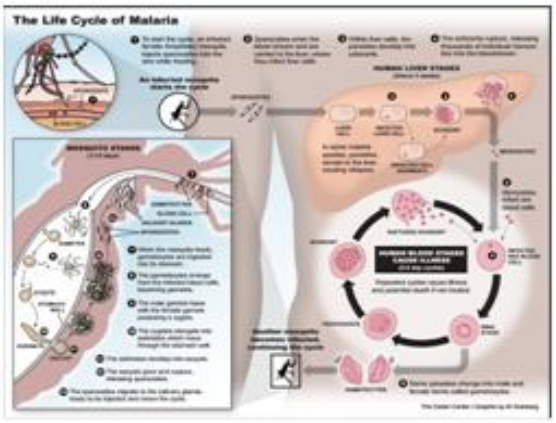

Figure 2: Malaria cycle

Figure 3: *Anopheles subpictus*

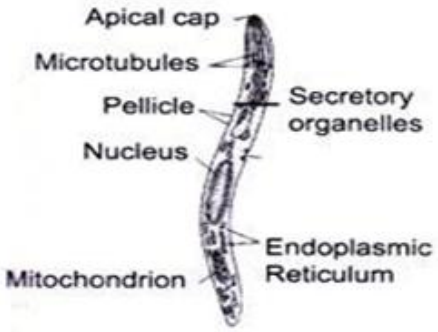

Figure 4: Sporozoite of *Plasmodium vivax*

The major vectors for spreading malaria, yellow fever, dengue fever, schistosomiasis, Japanese encephalitis (JE) and filariasis are mosquitoes. *Anopheles subpictus* Grassi and *Anopheles culicifacies* are major vectors of malaria, which are widely distributed in India, Afghanistan, Borneo, China, Malaysia, Philippines, Sri Lanka [11,12]. Now-a-days, people are suffering from Dengue very frequently. So, awareness should be created among the people for the prevention and management of dengue. The main vector of Dengue and Dengue haemorrhagic fever (DHF) is *Aedes aegypti*, belonging to *Aedes* genera. It has become quite difficult to manage the resistance to conventional insecticides. According to WHO, the main hindrance in preventing vector-borne diseases was resistance in vectors. The larval stage is a crucial target in the mosquito control programs. Many ill effects are caused by the use of synthetic organic insecticides, so natural, biodegradable and eco-friendly methods should be used for preventing dengue

2. Materials and Methods

Mosquito Larvae Culture

The larvae were cultured *in-vivo* in the wild, close to a bush in a fenced compound with plantain Vegetation, located at Bhojipura in Bareilly, U.P., India. The larva culture was carried out using standard methods as described by Ohimain et al [3].

Experimental Setup: The experimental setup was done to verify the larvicidal activity of the extract against the larvae. The positive control was adjusted with 1 ppm Pyrethrum pesticide, while the negative control was taken from water from the breeding site [3].

Plant Collection and Extraction

The leaf and stem of *Ocimum sanctum* were collected from Herbal garden, Shri Ram Murti Smarak, College of

Engineering and Technology (Pharmacy), Bareilly, U.P., India in September, 2016. The leaves and stem were shade-dried for 7 days at temperatures (30°C). About 300 g of the leaves and stem were powdered and macerated in 500 ml of ethanol for 72 hours. The extract was prepared by using soxhlet apparatus and was preserved at 4°C [3].

Preparation of plant extracts:

The leaf and stem were dried for 10-15 days in the shade at the temperatures (27-37^{o}C day time). The dried stem (500 g) and leaf (600 g) were powdered mechanically by electrical stainless steel blender and extracted with ethanol in a soxhlet apparatus (boiling point range 60–80°C) for 7 h. The extracts were filtered by funnel with Whatman filter paper. The extract was concentrated under reduced pressure 22-26 mm Hg at 45^{o}C and the extract residue obtained was stored at 4^{o}C. The residue was then made in to a 1 % stock solution with ethanol (stock solution). From the stock solution, 1000-4.69 mg/l, dilutions were prepared with distilled water. Polysorbate 80 was taken as an emulsifier at the concentration of 0.05 per cent in the final test solution.

Mosquito culture:

Anopheles subpictus and *Culex tritaeniorhynchus* larvae were collected from a field and stagnant water areas of Bhojipura and to start the colony and larvae were kept in plastic trays containing tap water. They were maintained and all the experiments were carried out at 28 ± 2°C and 75–85% relative humidity under 14:10 h light and dark cycles. Larvae were fed a diet of Brewer's yeast and algae collected from ponds in a ratio of 3.5:1.5.

Larvicidal bioassay:

For the bioassay experiment, larvae were taken in five batches of 20 in 249 ml of water and 1.0 ml of the plant extract concentration. The control was set up with ethanol and polysorbate 80. The numbers of dead larvae were counted after 24 h of exposure and the percentage of mortality was reported from the average of five replicates. 100% mortality was observed in leaf and stem ethanolic extract of *Ocimum sanctum* at concentration of (500, 250, 125, 62.5, 31.25, 15.63 and 7 mg/l) against *Anopheles subpictus and Culex tritaeniorhynchus*.

Statistical Analysis: The mean mortality and standard deviation of data from the experiment were calculated, to analyze the median lethal concentration [3].

3. Results and Discussion

The activity of plant extracts is often due to the complex of active compounds. Ethanolic extract was tested against *Anopheles subpictus* and *Culex tritaeniorhynchus* and 100 per cent larval mortality was observed in the leaf and stem ethanolic extract of *Ocimum sanctum*. The plant extracts showed moderate toxic effect on *Anopheles subpictus* and *Culex tritaeniorhynchus* after 24 h of exposure at 1000 mg/l.

Crude extracts of leaves or bark of the plants have been tested earlier by several investigators and larvicidal and antifeedent activities have been shown against *Anopheles subpictus* and *Culex tritaeniorhynchus.* It is concluded from the study that leaf and bark extract of *Ocimum sanctum* can be used as ecofriendly larvicides.

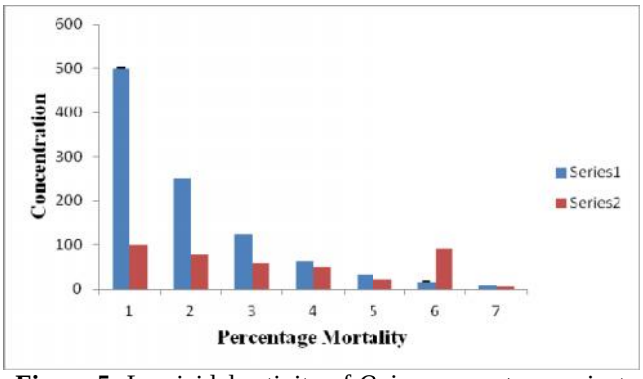

Figure 5: Larvicidal activity of *Ocimum sanctum* against *Anopheles subpictus*

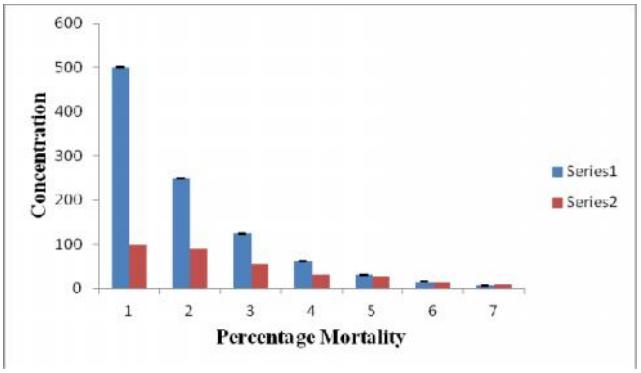

Figure 6: Larvicidal activity of *Ocimum sanctum* against *Culex tritaeniorhynchus*

Table 1: Different concentrations of *Ocimum sanctum against Anopheles subpictus* and *Culex tritaeniorhynchus* (mg/l) for larvicidal activity.

Concentration (mg/l)	Percentage mortality	
	Anopheles subpictus	***Culex tritaeniorhynchus***
500	99.25±0.60	99.25±0.60
250	79.5±0.70	90±0.53
125	60±0.53	55.58±0.84
62.5	50±0.53	31.75±0.49
31.25	20±0.73	25.58±0.84
15.63	90±0.53	15.5±0.84
7.82	5.16±0.55	9.08±0.47

4. Conclusion

In conclusion, our findings showed that leaf and bark extract of *Ocimum sanctum* can be used as efficient larvicide. The study also proved that leaf and bark extract of *Ocimum sanctum* can be used in the control and management of *Anopheles subpictus* and *Culex tritaeniorhynchus*. There is a possibility for further investigations of the efficacy of larvicidal properties of natural product extracts.

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