Mosquitocidal and water purification properties of *Ocimum sanctum* and *Phyllanthus emblica*

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Abstract

Ocimum sanctum was tested for its larvicidal and water sedimentation properties; the fruit ethanol and methanol extracts of *Phyllanthus emblica* were tested for phytochemical, larvicidal, oviposition-deterrent and ovicidal activities. Results emphasized that plant extracts have high toxicity against the egg and larvae of the malarial vector *Anopheles stephensi* and also have water sedimentation properties. LC_{50} of *Phyllanthus emblica* against *Anopheles stephensi* larvae ranged from 33.08 ppm to 81.26 ppm and from 23.44 to 54.19 ppm for ethanol and methanol extracts, respectively. *Phyllanthus emblica* also showed excellent ovipositional deterrent and ovicidal activities. The oviposition activity index value of ethanol and methanol extracts of *Phyllanthus emblica* at 500 ppm were -0.80 and -0.92, respectively. *Ocimum sanctum* includes both insecticidal secondary compounds, amino acids (glycine, lysine), vitamin C and other substances, that make treated water suitable for human consumption. Water quality parameters such as color, turbidity and pH were analyzed in the water samples (pre-treatment and post-treatment of plant extracts) taken from the breeding sites of mosquitoes. Hence, the plant product can be used as both mosquitocidal and water purifier.

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Introduction

Mosquitoes are the most single group of insects in terms of public health significance and transmitting dreaded diseases like malaria, filariasis and dengue, etc. There are approximately 460 recognized species. While over 100 can transmit human malaria, only 30-40 commonly transmit parasites of the genus *Plasmodium* that causes malaria which affects humans in endemic areas. The known vectors of *Anopheles* species, which are common in India, include *A. stephensi*, *A. culicifacies, A. fluviattis, A. minimmus, A. sudanicus* and *A. philippinensis. Anopheles stephensi* Liston, 1901 (Diptera: Culicidae) is the most common human-biting malaria vector in India and many West Asian countries, and is likely responsible for 40-50% of the annual malarial incidence (Curtis, 1994).

The problems of high cost, environmental risks and development of resistance in many vector mosquito species to several synthetic insecticides have revived interest in exploiting the pest control potential of plants (Grainage & Ahamed, 1988). Conventional water treatment relies on the addition of chemicals such as alum (aluminum sulfate) as coagulants and the addition of chlorine as a bactericide. The availability of these chemicals, which depends on foreign exchange, is unreliable and unpredictable. Because of economic and political constraints, the universal provision of piped water is not currently feasible, leaving millions of people without access to safe drinking water (WHO, 2005). This led us to look for plants with water purification properties.

Ocimum sanctum (holi basil), also called Tulsi in India, is ubiquitous in Indian tradition. Tulsi is described as a sacred medicinal plant in ancient literature (Kirtikar & Basu, 1975). It has been used to treat malarial fevers, ringworms, and other cutaneous afflictions (Butani, 1982). A variety of biologically active compounds have been isolated from the leaves, including ursolic acid, apigenin and luteolin. Some other main chemical constituents of Tulsi are Oleanolic acid, Rosmarinic acid, Eugenol, Carvacrol, Linalool, and β-caryophyllene (Merrily & Winston, 2007). This paper, therefore, describes the mosquitocidal and water sedimentation properties of *Ocimum sanctum* against malarial vector, *Anopheles stephensi.*

The plant genus *Phyllanthus* L. (Euphorbiaceae) is widely distributed in most tropical and subtropical countries. It is a very large genus consisting of approximately 550 to 750 species and is subdivided into ten or eleven subgenera. *Phyllanthus emblica L.* has been used for the

anti-inflammatory and anti-pyretic treatments by the rural population and for the treatment of several disorders such as the Scurvy, Cancer and Heart diseases. The important constituent of plant leaves has anti-neutrophilic activity and anti-platelet properties *in vitro*. The extracts also have several pharmacological properties, such as anti-viral (HIV, AIDS, herpes virus, cytomegalovirus) antimutagenic, antiallergic, anti-bacterial activities (Khopde *et al.,* 2000). *Phyllanthus emblica L.* contains a different class of secondary metabolites (Calixto *et al*., 1998).

The present study aimed to evaluate the effect of mosquitocidal and water sedimentation properties of *Ocimum sanctum* on the malaria vector *Anopheles stephensi* and screen phytochemicals from *Phyllanthus emblica L.* for larvicidal, pupicidal, oviposition deterrent and ovicidal effect against the same malaria vector.

Materials and methods

Colonization, mosquito rearing and maintenance

The eggs of *Anopheles stephensi* Liston, 1911 were collected from various water sources (*e.g.*, overhead tanks) in Coimbatore district, Tamil Nadu, India. The eggs were brought to the laboratory and were transferred to 18×13×4 cm size enamel trays containing 500 mL of water until they hatched. After hatching, the larvae were provided with dog biscuits and yeast at a 3:1 ratio and maintained at 27+2°C, 75-85% RH, under 14 h light (L): 10 h dark (D) photoperiod cycles. Pupae were collected and transferred to plastic jars (12×12 cm) containing 500 mL of water, which were placed in 90×90×90 cm screened mosquito cage for adult emergence. The freshly emerged adults were maintained at 27±2°C, 75-85% RH, under 14 h L: 10 h D photoperiod cycles. The adults were provided a 20% sugar solution *ad libitum*, and provided with rabbits (each exposed on the dorsal side) for 30 min two times per week (Hardstone *et al*., 2009). The males were provided with a 10% glucose solution on cotton wicks that was changed daily. Blood-fed females were provided filter paper-lined cups containing water for oviposition.

Collection of plant material and preparation of extracts

The leaves of *Ocimum sanctum* and *Phyllanthus emblica* fruits were collected from in and around Bharathiar University Campus, Coimbatore, Tamil Nadu, India. A total of 250 g of fresh, mature leaves was rinsed with distilled water and dried in a shade. The dried leaves were put in a Soxhlet apparatus (Borosil Glass Workers Ltd., Worli, Mumbai, India), and extracts were prepared using 100% ethanol (Loba Chemie Pvt. Ltd., Mumbai, India; 99 % purity) for 72 h at 30-40°C. Dried residues were obtained from 100 g of extract evaporated to dryness in a rotary vacuum evaporator. Two grams of the residues were dissolved in 100 mL of ethanol (2% stock solution), from which the following concentrations were prepared: 0.5%, 0.1%, 2.0%, 4.0%, and 8.0%, using distilled water.

Amla fruits were cut into small pieces and ground into a uniform powder using a blender. The methanolic extract of amla was prepared by soaking 100 g of dried powdered samples in 250 mL of methanol for 12 h. The extracts were filtered by using Whatman n. 1 filter paper. The filtrate was used for phytochemical screening. The preliminary qualitative phytochemical studies were performed for testing the different chemical groups present in fruit methanol extract of *Phyllanthus emblica* (Trease *et al.,* 1978; Kokate *et al.,* 1990).

Laboratory plant extract toxicity tests

F1-F2 larvae/pupae from the wild adult collection were used to evaluate larvicidal activity. Twenty-five larvae (in stages I to IV and pupae) were placed into a 500-mL glass beaker containing 249 mL of dechlorinated water and 1 mL of desired concentrations of plant extract. Test larvae and pupae were provided with food as previously described. At each tested concentration, 2-5 trials of 3 replicates were conducted concurrently. Control groups of larvae exposed to ethanol served as control. Mortality was corrected using Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \text{ X } 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae/pupae}}{\text{Number of larvae/pupae introduced}} \text{ X } 100$$

Ovicidal activity assay

For ovicidal activity, the freshly laid eggs were collected by providing ovitraps in mosquito cages. Ovitraps were kept in the cages two days after the female mosquitoes had been given a blood meal. The eggs were laid on the filter paper lining provided in the ovitrap. After scoring, 100 gravids were placed in a screen cage where ten oviposition cups were introduced for oviposition 30 min before the start of the dusk period. Of these ten cups, eight were each filled with test solution of 150, 200, 250, 300, 350, 400, 450, 500 ppm, and one was filled with 100 mL of respective solvent containing water and Polysorbate 80 that served as a control. A minimum of 100 eggs were used for each treatment, and the experiment was replicated 5 times. After treatment, the eggs were sieved through muslin cloth, thoroughly rinsed with tap water, and left in plastic cubs filled with dechlorinated water for hatching assessment after counting the eggs under the microscope (Su & Mulla, 1998). The percent egg mortality was calculated on the basis of non-hatchability of eggs with unopened opercula (Chenniappan & Kadarkarai, 2008). The hatching rate of eggs was assessed after 98 h post-treatment according to the method of Rajkumar and Jebanesan (2009).

Oviposition deterrence activity

To study the ovipositional deterrence effect and the number of eggs deposited in the presence of extracts with different solvents of experimental plants, a multiple concentration test was carried out. For bioassay test, 20 males and 20 females were separated in the pupal stage (by size of the pupae) and were introduced into screen cages (45×45×40 cm) in a room at 27±2°C and 75-85% relative humidity (RH) with a photoperiod of 14:10 h light and dark cycles. The pupae were allowed to emerge to adults in the test cages. Adults were provided continuously with 10% sucrose solution in a plastic cup with a cotton wick. They were blood fed (from pigeon) on Day 5 after emergence. In the multiple concentration test, five cups, each containing 100 mL distilled water with a 9-cm piece of white filter paper for oviposition, as well as solvent extracts at concentrations of 100, 200, 300, 400 and 500 ppm, were placed in each cage. A sixth cup without extract served as a control. The positions of the plastic cups were alternated between the different replicates so as to nullify any effect of position on oviposition. Five replicates for each concentration were run with cages placed side by side for each bioassay. After 24 h, the number of eggs laid in the treated and control cups were counted under a stereomicroscope. The percent effective repellency for each concentration was calculated using the following formula:

$$ER\ (\%) = \frac{NC-NT}{NC} \times 100$$

where ER is effective repellency, NC is number of eggs in control, and NT is number of eggs in treatment (Rajkumar & Jebanesan, 2009). The oviposition experiments were expressed as mean number of eggs and oviposition activity index, which was calculated using the following formula:

$$OAI = \frac{NT - NS}{NT + NS}$$

where NT is total number of eggs in the test solution and NS is total number of eggs in the control solution. Oviposition active index of +0.3 and above are considered as attractants while those with –0.3 and below are considered as repellents (Kramer & Mulla, 1979). Positive values indicate that more eggs were deposited in the test cups than in the control cups and that the test solutions were attractive. Conversely, negative values indicate that more eggs were deposited in the control cups than in the test cups and that the test solutions were a deterrent.

Field trials of plant extract larval toxicity

Plant extracts formulations (51 g*l-1) were applied to the water surface with a knapsack sprayer (Ignition Products Ltd., India, 2008). Pretreatment and post-treatment at 24, 48 and 72 h was conducted using a larval dipper. Larvicidal efficacy was carried out against late third and early fourth-instar larvae. Larvae were identified and counted to determine the relative species composition of each test site. Six trials were conducted for each test site (standing water bodies) with similar weather conditions (27°C; 79% RH). The required quantity of plant extract was determined by calculating the total surface area and volume (0.25 m^2 and 250 L). The required concentration was prepared using 10 times the observed laboratory LC_{50} values (Murugan *et al*., 2003). Percentage reduction of the larval density was calculated using the formula:

$$Percentage\ reduction = \frac{C - T}{C} \times 100$$

where C is the total number of mosquitoes in control and T is the total number of mosquitoes in treatment.

Water quality parameters

Water quality parameters such as pH, color and turbidity were investigated using the methods of Clescerl *et al.* (2005). To prepare the coagulants and treatment (Schwarz, 2000), leaves of *Ocimum sanctum* were shade-dried, powdered using an electric blender, and mixed with a small amount of clean water to form a paste. The paste was diluted to the required strength based on raw water turbidity. Total suspended solids in raw water separated as over 50, between 50 and 150, and over 150 mg*l-1, and the final concentration used for treatment was 50, 30-100 and over 150 mg*l- 1, respectively (Schwarz, 2000). After filtering insoluble material with a fine-mesh screen or muslin cloth, the coagulant was added and stirred fast for 30 s. The treated water was then covered for 1 h without disturbance.

Statistical analysis

The SPSS (Version 9.0) software package was used to analyze data obtained from the bioassay. Lethal concentrations (LC), LC_{50} and LC_{90}, Duncan Multiple Range Test) and χ^2 tests were used.

Results

Ocimum sanctum leaf extract was used to test water purification properties including water color, total suspended solids and pH, and was effective in sedimentation and purification. Before treatment, water color was 31 HU while after this was 12 HU. Total suspended solids before treatment was 40.0 mg*l-1 and this was reduced 30.0 mg*l-1, after. Similarly, pH level was 8 before treatment and 6.8 after.

Significant mortality was evident after the treatment of *Ocimum sanctum* leaf extract (OSLE) at different concentrations against *A. stephensi* in laboratory (Table 1). After the treatment of OSLE at different concentration levels (0.5-8%), 38% mortality was noted at I instar larvae by the treatment of OSLE at 0.5%, whereas this increased to 90% at 8% of OSLE treatment. Mortality increased with increasing concentration. Lethal concentrations (LC_{50} and LC_{90}) were also calculated. The LC_{50} and LC_{90} values are represented as follows: LC_{50} value of I instar was 1.52%, II instar was 2.22%, III instar was 3.11%, IV instar was 5.13% and pupae was 6.45%. LC_{90} value of I instar was 7.39%, II instar was 8.68%, III instar was 10.07%, IV instar was 14.11% and pupae was 15.24%, respectively.

An. stephensi larvae were collected exclusively in overhead water tanks and mean larval count was calculated. Breeding sites treated with *O. sanctum* extracts, showed a reduction in *An. stephensi* larvae. The field experiment was carried out at drinking water tanks (0.5×0.5×1.0) with *Anopheles stephensi* larvae and the percentage reduction/mortality was 82.5%, 87.9% and 92.4% after the 24 h, 48 h and 72 h, respectively (Table 2).

Table 3 shows qualitative analyses of the fruit extract of *Phyllanthus emblica,* emphasizing the presence of proteins, tannins and terpenoids. Steroids were absent in *Phyllanthus emblica.*

The larvicidal and pupicidal activities of the ethanol extract of *Phyllanthus emblica* against *Anopheles stephensi* larvae under laborato-

Table 1. Larvicidal activity of *Ocimum sanctum* against malaria vector, *Anopheles stephensi*.

Larval instars and Pupa	Larval mortality (%)±SD Concentration of *Ocimum sanctum* (%) 0.5	1.0	2.0	4.0	8.0	LC_{50} (LC_{90})	95% Confidence limit LCL LC_{50} (LC_{90})	UCL LC_{50} (LC_{90})	Chi-square value
I	38±0.6[a]	43±0.3[ab]	58±1.2[b]	75±0.5[c]	90±0.8[d]	1.52067 (7.38616)	0.85569 (2.07067)	6.34024 (8.99671)	2.830*
II	32±0.9[a]	41±0.5[b]	50±1.0[c]	69±0.7[d]	85±1.1[e]	2.21933 (8.68104)	1.57467 (2.80353)	7.42155 (10.65323)	2.769*
III	26±1.0[a]	35±1.2[b]	46±1.4[c]	61±0.8[d]	79±1.5[e]	3.10581 (10.06631)	2.47306 (3.76193)	8.56234 12.46361	3.384*
IV	19±1.5[a]	25±0.8[ab]	39±0.6[b]	52±1.2[c]	61±0.4[d]	5.12732 (14.11467)	2.94904 (13.82370)	9.02274 (56.49114)	8.278*
Pupa	10±0.5[a]	21±1.0[b]	32±1.2[c]	46±0.8[d]	53±0.6[e]	6.44729 (15.23744)	3.67191 570.79738	9.00805 (2199.74679)	13.287*

Within a column means followed by the same letter(s) are not significantly different at 5% level by Duncan Multiple Range Test; *Significant at P<0.001 (heterogeneity factor used in calculation of confidence limits). SD, standard deviation; LC_{50}, LC_{90}, lethal concentration; LCL, lower confidence limits; UCL, upper confidence limits.

ry conditions are given in Table 4. Percentage mortality was 43% at 20 ppm concentration and increased to 82% at 100 ppm concentration against the first instar larvae. Median lethal concentrations (33.08, 48.85, 68.28, 81.26 and 86.24 ppm) for larvae and pupae were low and significant.

Table 5 shows larvicidal and pupicidal activities of methanol extract of *Phyllanthus emblica* against the malarial vector, *Anopheles stephensi.* Among the four larval stages, I Instar larvae were more susceptible than the other instars. The fruit extracts also showed considerable pupal mortality. The lowest mortality observed was 20% at 20 ppm against pupae and the highest was 98% at 100 ppm against the I instar larvae. LC_{50} (23.44, 33.10, 42.13, 54.19 and 64.28 ppm for four instar larvae and pupae, respectively) observed for larvae and pupae were very low when compared to the ethanol extract.

In the oviposition deterrent assay, gravid *Anopheles stephensi* preferred to lay eggs in the distilled water control cups than in the cups treated with solvent extracts of *Phyllanthus emblica* (Table 6). There was also a marked difference in the number of eggs laid. Observed results showed that the 500 ppm treated cups received a mean number of 49±1.15 and 19±1.53 eggs per cup in fruit ethanol and methanol extracts of *Phyllanthus emblica* treatment while the control cups received a mean number of 456±1.50 and 470±1.30 eggs per cup. The present results indicated that the oviposition deterrence was concentration dependent, as 500 ppm of ethanol and methanol fruit extracts of experimental plant exhibits strong deterrent effect when compared with 100 ppm against oviposition. The solvent leaf extracts strongly deterred oviposition by gravid *Anopheles stephensi*, with a significantly lower proportion of eggs being laid on ovitraps containing extracts in comparison with control solutions ($P<0.05$). The maximum percentage of effective repellency against oviposition was 96.93%, reported in 500 ppm followed by 95.95, 94.13, 87.01, 78.18 and 67.50% at 500, 400, 300, 200 and 100 ppm methanol extracts of *Phyllanthus emblica*, respectively. The percentages of egg hatchability of *Anopheles stephensi* with the fruit ethanol and methanol extracts of *Phyllanthus emblica* are presented in Table 7. The ethanol and methanol extracts of *Phyllanthus emblica* exerted 100% mortality (no hatchability) at 400 ppm and above. Very low hatchability (19±1.20% and 0%) was observed at a 350 ppm concentration of ethanol and methanol extracts of *Phyllanthus emblica*, respectively against *Anopheles stephensi*. Almost 100% hatchability was obtained in the control. In the case of ovicidal activity, exposure to freshly laid eggs was more effective than to the older eggs.

Table 2. Effect of *Ocimum sanctum* treatments of drinkng water tanks malarial vector, *Anopheles stephensi*.

Site no.	Larval density (%)			
	Before treatment	After treatment		
		24 h	48 h	72 h
1	79	19	15	11
2	71	14	12	8
3	65	10	8	5
4	57	7	6	3
5	47	7	2	0
6	39	4	0	0
Total	358	61	43	27
Average	59.6	10.1	7.16	4.50
% Reduction	-	82.50	87.9	92.4

-Place: Vadavalli;
-Habitat: drinking water;
-Size: 0.5×0.5×1.0;
-Depth: 1 cm;
-Species: *Anopheles stephensi*;
-Stage: larvae stage;
-Calculation: 2.5×1.5 m; 3.10×1=31.00.

Table 3. Phytochemical constituents present in *Phyllanthus emblica*.

Phyto-constituents	Ethanol extract	Methanol extract
Flavonoidds	+	+
Tannins	+	+
Carbohydrates	+	+
Alkaloids	+	+
Proteins	+	+
Steroids	–	–
Terpenoids	+	+

Discussion and conclusions

Many approaches have been developed to control the mosquito menace. One such approach to prevent mosquito-borne disease is by killing mosquito at the larval stage. The current mosquito control approach is based on synthetic insecticides. Even though they are effective, they created many problems, such as insecticide resistance (Liu *et al.,* 2005), pollution, and toxic side effects on humans (Lixin, 2006). In the present study, OSLE showed higher larvicidal activities against mosquito probably due to the presence of active compounds such as eugenol and (E)-6-hydroxy-4,6-dimethyl-3-heptene-2-one (Kelm & Nair, 1998). The mosquito larvicidal property of leaf and flower extracts of *Ocimum sanctum* L. against *Aedes aegypti* and *Culex quinquefasciatus* larvae has been previously reported (Anees, 2008). Mosquito breeding habitats vary from ponds, marshes, ditches, pools, drains, water containers and other similar water collections, and are often species-specific (Rozendaal, 1997). The increase in the mosquito vector population and the incidence of mosquito-borne diseases (*e.g.*, malaria, dengue, and Chikungunya) is rising in India as a result of inadequate water supply systems and contamination. Storage of water, often from untreated water sources, and polluted water systems serve breeding sites of mosquitoes that transmit mosquito-borne pathogens, in addition to water-borne pathogens (*e.g.*, cholera, dysentery and typhoid) (Vinod, 2011). In the present study, the treatment with coagulants at the breeding sites of mosquito had not only killed mosquito larvae but it had also water purifying properties. Biopesticide spray operations had been performed in the past (Murugan, 2006) for the control of vectors in the tsunami affected areas of India and the plant products such as neem and other herbal combinations showed biopesticidal potency killing mosquito larvae in the contaminated water. Larvicidal effect of neem (*Azadirachta indica*) oil cake was studied against mosquitoes. The oil cake showed good larvicidal activity against the mosquito species tested (Shanmugasundaram *et al.,* 2008). Neem is derived from the neem tree *Azadirachta indica* A. Juss. (Meliaceae), and its primary insecticidal components are the tetranortriterpenoid, azadirachtin and other limonoids. The effect of neem limonoids azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione and deacetylnimbin on insects was investigated by Senthil Nathan *et al.* (2006).

Coagulants of *Ocimum sanctum* were tested for the purifying properties at the laboratory by adding coagulants with waters from mosquito breeding sites and coagulants also had water purifying properties; treated water showed cleaning efficacy. Plant product nutrients (vitamin A and C, calcium, iron and zinc) and allelochemicals (Tannins, alkaloids, glycosides and saponins) not only removed solid contami-

Table 4. Toxicity evaluation of ethanol extract of *Phyllanthus emblica* against the malarial vector *Anopheles stephensi*.

Larval stages	% of larval mortality Concentration (ppm) 20	40	60	80	100	LC_{50} (LC_{90})	Regression equation	95% Confidence limit LCL LC_{50} (LC_{90})	UCL LC_{50} (LC_{90})	Chi-square value
I	43±1.32[a]	52±1.80[b]	68±2.59[c]	71±0.5[cd]	82±1.80[d]	33.08 (128.48)	Y=-0.44449 +0.01343X	18.26 (110.01)	42.76 (162.10)	1.125
II	36±0.70[a]	43±1.22[b]	59±1.14[c]	62±1.41[cd]	78±0.79[d]	48.85 (142.98)	Y=-0.66503 +0.01361X	38.55 (122.19)	57.18 (180.48)	1.793
III	20±0.65[a]	37±0.79[b]	48±1.58[c]	52±1.51[cd]	70±0.79[d]	68.28 (151.79)	Y=-1.04788 +0.01535X	60.85 (131.41)	76.82 (186.42)	2.670
IV	16±0.35[a]	23±0.79[b]	33±1.11[c]	44±1.81[d]	68±1.06[e]	81.26 (153.33)	Y=-1.44506 +0.01778X	74.16 (134.81)	90.58 (183.07)	2.728
Pupa	12±1.59[a]	21±0.65[b]	31±1.19[c]	40±1.98[d]	64±1.06[e]	86.24 (156.45)	Y=-1.57407 +0.01825X	78.85 (137.64)	96.30 (186.67)	2.032

Within a column means followed by the same letter(s) are not significantly different at 5% level by Duncan Multiple Range Test. LC_{50}, LC_{90}, lethal concentration; LCL, lower confidence limits; UCL, upper confidence limits.

Table 5. Toxicity evaluation of methanol extract of *Phyllanthus emblica* against the malarial vector *Anopheles stephensi*.

Larval stages	% of larval mortality Concentration (ppm) 20	40	60	80	100	LC_{50} (LC_{90})	Regression equation	95% Confidence limit LCL LC_{50} (LC_{90})	UCL LC_{50} (LC_{90})	Chi-square value
I	49±0.70[a]	65±0.79[b]	75±0.35[c]	87±0.79[d]	98±1.27[e]	23.44 (82.15)	Y=-0.51187 +0.02183X	12.98 (74.12)	30.87 (93.77)	3.173
II	40±0.54[a]	58±0.79[b]	69±1.06[c]	82±1.08[d]	97±1.29[e]	33.10 (89.81)	Y=-0.74820 +0.02260X	25.11 (81.53)	39.27 (101.65)	4.043
III	38±0.35[a]	44±0.70[ab]	61±1.22[b]	76±1.41[c]	90±1.76[d]	42.13 (107.52)	Y=-0.82596 +0.01960X	34.42 (96.59)	48.39 (123.90)	2.881
IV	29±0.74[a]	37±0.65[b]	53±1.51[c]	62±1.29[d]	88±1.38[e]	54.19 (119.26)	Y=-1.06730 +0.01969X	36.01 (95.48)	69.28 (187.81)	6.398
Pupa	20±0.35[a]	29±0.70[b]	48±0.93[c]	52±1.47[cd]	85±1.88[d]	64.28 (124.56)	Y=-1.36664 +0.02126X	46.70 (97.90)	85.52 (216.27)	9.100

Within a column means followed by the same letter(s) are not significantly different at 5% level by Duncan Multiple Range Test. LC_{50}, LC_{90}, lethal concentration; LCL, lower confidence limits; UCL, upper confidence limits.

Table 6. Oviposition deterrence activity of ethanol and methanol extracts of *Phyllanthus emblica* against the malarial vector *Anopheles stephensi*.

Concentration (ppm)	Ethanol Number of eggs±S.E. Treatment	Control	ER%	OAI	Methanol Number of eggs±S.E. Treatment	Control	ER%	OAI
500	49±1.15[a]	456±1.50	89.25	-0.80	19±1.53[a]	470±1.30	95.95	-0.92
400	54±1.41[b]	389±1.71	86.11	-0.75	23±1.22[ab]	392±1.84	94.13	-0.88
300	62±1.72[c]	321±1.20	80.68	-0.67	37±1.84[b]	285±1.61	87.01	-0.77
200	86±1.01[d]	266±1.41	67.66	-0.51	48±1.73[c]	220±1.42	78.18	-0.64
100	98±1.52[e]	210±1.47	53.33	-0.36	52±1.32[cd]	160±1.32	67.50	-0.50

Within a column means followed by the same letter(s) are not significantly different at 5% level by Duncan Multiple Range Test. S.E., standard error; ER, effective repellency; OAI, oviposition active index.

Table 7. Ovicidal activity of ethanol and methanol extracts of *Phyllanthus emblica* against eggs of *Anopheles stephensi*.

Treatment	Extract	Percentage of egg hatchability±S.D. Concentration of extract (ppm) 150	200	250	300	350	400	450	500	Control
Phyllanthus emblica	Ethanol	96±1.24[e]	71±2.43[d]	54±1.40[c]	30±2.49[b]	19±1.20[a]	NH	NH	NH	100±0.00
	Methanol	74±2.17[d]	47±1.71[c]	22±2.10[b]	14±1.03[a]	NH	NH	NH	NH	98±2.95

Within a column means followed by the same letter(s) are not significantly different at 5% level by Duncan Multiple Range Test. S.D., standard deviation; NH, no hatchability (100% mortality).

nants, but also greatly reduced amounts of harmful bacteria in the waste water. Earlier studies examined the antimicrobial property present in the *Ocimum sanctum*, and concluded that the component responsible was likely to be eugenol. This component has been demonstrated to have both antibacterial (Nakaruma *et al.,* 1999) and antihelmintic activities (Pessoa *et al.,* 2002). Nareshkumar *et al.* (2011) reported mosquitocidal and water purifying properties of different plant extracts (*Cynodon dactylon*, *Aloe vera*, *Hemidesmus indicus* and *Coleus amboinicus*) on various mosquito vectors (*Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*) at different water samples.

In the present study, we also sought to determine whether methanol and ethanol extracts from *Phyllanthus emblica* fruits could be used for mosquito control. We observed a functional response by all immature life stages of *A. stephensi* to ethanolic and methanolic extracts of *Phyllanthus emblica* fruits. This biological activity is attributed to the compounds present in fruits, including flavonoids, phenols, and steroids that together or independently result in morbidity and mortality in *A. stephensi*. Park *et al.* (2000) showed that the biological activity of the plant extracts might be due to the various compounds, including phenolics, terpenoids and alkaloids existing in plants. These compounds may jointly or independently contribute to produce larvicidal activity against mosquitoes. A piperidine alkaloid from Piper longum fruit was found to be active against mosquito larvae of *Cx. pipiens* (Lee, 2000).

It was recognized that the fourth larval stage of mosquitoes was more tolerant to toxicant then early instars (Mulla, 1961; Rettich, 1976; Nareshkumar *et al.,* 2012). Larval mortality may be due to the effect of chemicals like flavonoids, alkaloids, and terpenoids. The higher mortality of mosquito larvae was due to the combined action of plant compounds that might be acting on the midgut epithelium cells exerting their toxic effects on mosquito. Lethal LC_{50} observed in the present study is very low when compared to the earlier studies. The mangrove plant *Rhizophora mucronata* bark and pith extract showed toxicity with LC_{50} values of 157.4 and 168.3 ppm, respectively, against *Ae. aegypti* larvae (Kabaru & Gichia, 2001).

Exposure of *A. stephensi* larvae to sub-lethal doses of neem extracts in the laboratory, prolonged larval development, reduced pupal weight, caused high oviposition deterrence and high mortality (Wandscheer *et al.,* 2004). *Phyllanthus emblica L.* was also effective in oviposition deterrence and ovicidal activities. Adult female *A. stephensi* avoided oviposition in *Phyllanthus emblica*-treated water, though some laid eggs, but these hatched in abnormal larvae. The ethanolic leaf extracts of *Cassia obtusifolia* at high concentration (400 mg*l-1) were responsible for 92.5% oviposition deterrence effect, while 300, 200 and 100 mg*l-1 were responsible for 87.2%, 83.0%, and 75.5% deterrence effect, respectively (Rajkumar & Jebanesan, 2009). The leaf extract of *Solanum trilobatum* reduced egg laying by gravid females of *Anopheles stephensi* from 18% to 99% compared with ethanol-treated controls at 0.01, 0.025, 0.05, 0.075, and 0.1% (Rajkumar & Jebanesan, 2005). Methanol extract of *Phyllanthus emblica* showed more deterrence and egg mortality than the ethanol extract. The crude acetone extract of *Cuscuta hyaline* was an effective oviposition deterrent against *Culex quinquefasciatus* at a concentration of 80 ppm (Mehra & Hiradhar, 2002). It has been shown that the age of the embryos at the time of treatment plays a crucial role with regard to the effectiveness of the chitin synthesis inhibitor dimilin to *Culex quinquefasciatus* (Miura *et al.,* 1976). The Ovicidal effect of Solenostemma argel was low; however, concentrations of 0.05 % and 0.1 % exhibited significant effects ($P<0.05$), producing 65-75% and 62.9-62.9%, respectively, on the first and second day after treatment, the 0.1% concentration reduced egg hatch by 33.7%, compared with control, and 100% mortality values were evident in concentrations as low as 0.025% at two days post-hatching against *Culex pipiens* (Al-Doghairi *et al.,* 2004). The seed extract of *Atriplex canescens* showed complete ovicidal at 1000 ppm concentration in eggs of *Culex quinquefasciatus*. The bioactive compound Azadirachtin isolated from *Azadirachta indica* showed complete Ovicidal activity in eggs of *Culex tarsalis* and *Culex quinquefasciatus* exposed to 10 ppm concentration (Murugan *et al.,* 1996; Ouda *et al.,* 1998).

The phytochemical analysis of *Phyllanthus emblica* L. reveals the presence of alkaloids, tannins and saponins. These compounds are known to be biologically active. Tannins have important roles such as stable and potent antioxidants (Trease *et al*., 1978). Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). That of the largest group of chemicals produced by plants are the alkaloids and their amazing effect on humans has led to the development of powerful pain killer medications (Raffauf, 1996). Fruits are an important group of foodstuffs in the diet. These components of human diet are not adequately replaceable by any other products. Plant tissues are naturally rich in nutritive or therapeutically active products of plant secondary metabolism. The consumption of fruits has been inversely associated with morbidity and mortality from degenerative diseases (Aruoma, 1998; Özen, 2010) and is associated with low incidences and mortality rates of cancer and heart disease (Ames *et al*., 1993; Dragsted *et al*., 1993). It is not known which dietary constituents are responsible for this association, but antioxidants appear to play a major role in the protective effects of plant foods (Gey 1990; Barberousse *et al*., 2008; Patra & Kumar, 2010). Fruit contains considerable amounts of active components such as polyphenols, flavonoids, tannins, vitamins A, B, C and E, and carotenoids which are considered potent scavengers of free radicals and reactive oxygen species (Rice-Evans *et al*., 1995).

The present results suggest that *Ocimum sanctum* can be used for mosquitocidal and water purifying purposes for promoting water sustainability in developing countries. *Ocimum sanctum* coagulum has an added advantage of having antimicrobial properties. Considering the fact that *Ocimum sanctum* coagulum can be locally produced, its use in water purification should be encouraged. This is likely to reduce the high cost of the current water treatment systems. Experiments on phytochemical screening of *Phyllanthus emblica* for their, larvicidal, pupicidal, ovipositiondeterrent and ovicidal activity have opened the possibility of further investigations on their efficiency, in view of the utilization of their higher biomass. However, the mechanism of action of the compounds from *Phyllanthus emblica* is still not clear. Studies on isolation, purification and the mechanism of action of individual compounds existing in the *Phyllanthus emblica* are needed and these are in progress.

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