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Agnimantha: An herbal larvicide and pupicide against malarial vector *Anopheles stephensi*

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Abstract
Malaria has 85% global infectious disease burden spread over 90 countries and territories in the tropical and subtropical regions. Worldwide next to Africa, in the South East Asia, India has the highest 77% malarial burden. Amid of various methods to control vector-borne diseases, vector control is a superior alternative. As larvicidal resistance is increasing among mosquitoes, herbal stratagems are forthcoming. In this study, antilarval and antipupal activities were carried out by using aqueous and ethanolic leaf extracts of *Clerodendrum phlomidis* against early fourth instar larvae and pupae of *Anopheles stephensi*. The result indicates that the aqueous and ethanolic leaf extract of *Clerodendrum phlomidis* have larvicidal and pupicidal activity as a green-collar approach for mosquito control.

Keywords: Malaria, *Anopheles stephensi,* antilarval, antipupal, *Clerodendrum phlomidis.*

1. Introduction
Among vector-borne diseases *viz*., dengue fever, chikungunya, filariasis and Japanese encephalitis, malaria is the most affected disease [1, 3]. With 85% global infectious disease burden, malaria is found in 90 countries and territories in the tropical and subtropical regions [1]. Malaria causes ~2-3 million cases worldwide including infants, children and adults [2]. Worldwide next to Africa, in the South East Asia, India has the highest 77% malarial burden [4] with Orissa, Jharkhand, West Bengal, North Eastern states, Chhattisgarh, and Madhya Pradesh most affected regions [5].

Most answerable vectors for malaria are mosquitoes which also responsible for mortality, angioedema, intense itching, redness of skin and swellings [6]. The predominant malarial vectors are *Anopheles culicifacies, Anopheles stephensi, Anopheles fluviatilis, Anopheles minimus, Anopheles dirus* and *Anopheles sundaicus* and secondary vectors reported are *Anopheles varuna, Anopheles annularis, Anopheles philippinensis, Anopheles jeyporiensis* and *Anopheles subpictus* [7]. *Anopheles stephensi* is mostly found in urban areas and rainfall, warm temperatures, stagnant clean water bodies and puddles which are the perfect habitats for mosquito larvae to multiply [6, 8]. Amid of various methods to prevent malaria, vector control is the globally accepted strategy to restrict the transmission of parasites [7]. The indoor residual spraying and long lasting insecticide treated bed nets [9], synthetic organic chemicals and larvicides [10] has been used for controlling mosquitoes. Some examples of larvicides are kerosine, paris green, chlordan, aldrin and Dieldrin [7]. *Anopheles stephensi* is resistant to organochlorine (DDT), Organophosphate (malathion) and Pyrethroid (deltamethrin) [7]. Anopheline mosquitoes resist the larvicides either by metabolic resistance by enhanced levels of detoxification enzymes like esterase or through target site insensitivity via modifications of receptors [11]. The resistance to chloroquine therapy has been increased in the India to both types of malaria, *Plasmodium vivax* and *Plasmodium falciparum* [5]. Also *Plasmodium falciparum* found to be resistant to antimalarial drug artemisinins [12]. *Plasmodium falciparum* has highest mortality rate followed by *Plasmodium vivax, Plasmodium malaria, Plasmodium ovale* and *Plasmodium knowlesi* [8].

Resistance to drugs as well as insecticides [8, 9] is the main hurdle to overcome the dream of malaria free country. To bridle these problems, safe and effective herbal stratagem is in focus against the vectors and vector borne diseases [13]. It has been proved by several studies [14, 17] that environment friendly and biodegradable natural insecticides [18] can successfully act as larvicides [19]. The plant *Clerodendrum phlomidis* Linn. F. (Synonym: *Clerodendrum multiflorum* (Burn. F.) O. Ktze) belongs to the genus *Clerodendrum* L. of the family

Verbenaceae with about 500 species found in tropical and subtropical regions of the world [20]. It is ordinarily known as agnimantha in Sanskrit, arni in Hindi, arani or tekar in Marathi [21]. It is substantially used in Ayurveda and Siddha system of medicine [22, 23] and owns anti-inflammatory, antiarthritic, analgesic, antiobesity, antihepatotoxic, hypoglycaemic [20, 21], antimicrobial and antifungal activities [24]. In the present study, the toxicity of leaf extracts of *Clerodendrum phlomidis* against the malarial vector *Anopheles stephensi* was evaluated.

2. Materials & Methods

2.1 Collection of Plant Materials

The *Clerodendrum phlomidis* taxonomically identified and affirmed. The fully developed, non-infected, healthy leaves were collected from the area around Swami Ramanand Teerth Marathwada University, Nanded and voucher specimen of the *Clerodendrum phlomidis* deposited at The Herbarium of School of Life sciences, S.R.T.M. University, Nanded, MS, India.

2.2 Preparation of Plant Extracts

The collected leaves were washed with tap water several times and air dried. Then the leaves were shade dried for 7-10 days at room temperature. The dried leaves were powdered using stainless steel blender. The finely ground plant leaf powder (25 gm/250 ml solvent) was loaded in Soxhlet apparatus and was extracted with distilled water and ethanol.

The crude extract were collected and filtered through Whatman filter paper number 1. The extracts were concentrated by evaporating to dryness. The residue obtained was stored in a refrigerator at 4 °C. One gram of residue of each extract was dissolved separately in 100 ml of acetone, as stock solution and then different concentrations, 60 to 200 mg/l; dilutions were prepared with dechlorinated tap water. Tween 20 was used as an emulsifier at the concentration of 0.02% in acetone in the final test solution.

2.3 Collection of Larvae

The larvae were collected from the stagnant water bodies, an ideal breeding site for mosquitoes, around the S.R.T.M. University, Nanded, MS, India. The fourth instar larvae of *Anopheles stephensi* were identified and separated on the basis of their microscopic and macroscopic features [25, 26]. The larvae were fed on Brewer's yeast, dog biscuits and algae in the ratio 3:1:1, respectively [27].

2.4 Larvicidal Activity

Anopheles stephensi was used to test the larvicidal activity of leaf extract of *Clerodendrum phlomidis*. The larvicidal activities were assessed by the procedure of WHO [12]. The larvae were transferred to small plastic jar and damaged or dead larvae were removed. For the bioassay test, 25 larvae were taken in in 249 ml of water and 1.0 ml of the desired plant extract concentration. The control was set up with acetone and tween 20. Experiments were carried out with a series of concentrations (as 60, 80, 100, 120, 140, 160, 180, 200 mg/l) each with three replicates for both aqueous and ethanolic extracts.

The numbers of dead larvae were counted after 24 hours of exposure and the percentage of Mortality was reported from the average of three replicates and LC_{50}, LC_{90} values calculated.

$$\% \text{ Mortality} = \frac{\% \text{ Test Mortality} - \% \text{ Control Mortality}}{100 - \% \text{ Control Mortality}} \times 100$$

2.5 Pupicidal Activity

From the maintained larvae, twenty five freshly emerged pupae were used for pupicidal activity. In bioassay, to 249 ml of water 1.0 ml of desired concentration of plant extract added. The control set up with acetone and tween 20. Experiments were carried out with a series of concentrations (as 60, 80, 100, 120, 140, 160, 180, 200 mg/l) each with three replicates for both aqueous and ethanolic extracts. The number of dead pupae counted after 24 hours of exposure and the percentage of mortality, LC_{50} and LC_{90} was reported from the average of three replicate.

2.6 Statistical analysis

The statistical analysis of the experimental values calculated using MS Excel 2010. $P< 0.05$ was considered to be statistically significant.

Table 1: % Mortality of larvae and pupae of *Anopheles stephensi* in aqueous and ethanolic extract of *Clerodendrum phlomidis*.

Sr. No.	Concentration mg/l	Control	60	80	100	120	140	160	180	200
1	% mortality of larvae in Aqueous extract	00	11.11	14.77	18.44	37	51.77	51.77	70.33	84.44
2	% mortality of larvae in Ethanol extract	00	29.55	33.33	59.22	66.66	88.88	92.55	100	100
3	% mortality of pupae in Aqueous extract	00	3.66	11.11	37.00	40.66	55.55	59.22	70.33	81.44
4	% mortality of pupae in Ethanol extract	00	8.25	29.12	37.50	54.12	58.25	66.62	83.25	100

3. Results

The antilarval activity of aqueous extracts of leaves of *Clerodendrum phlomidis* showed highest mortality of 84.44% at 200 mg/l while ethanolic extract showed 100% mortality at 180 & 200 mg/l concentrations. The aqueous and ethanolic extract of leaves of *Clerodendrum phlomidis* showed highest antipupal activity of 81.44% and 100% mortality at 200 mg/l concentrations respectively. (Table 1)

The aqueous and ethanolic extract of leaves of *Clerodendrum phlomidis* showed LC_{50} values as 150.32 & 90.39 mg/l respectively and LC_{90} values as 250.32 & 157.28 mg/l respectively for antilarval activity against *Anopheles stephensi* and their corresponding regression equations are y = 0.400x - 10.13& y = 0.598x - 4.057 respectively. Similarly for antipupal activity aqueous and ethanolic extract showed LC_{50} values are 139.95 & 121.30 mg/l respectively and LC_{90} values as 231.07 & 204.29 mg/l respectively with their corresponding regression equations $Y=0.0494_x$- 1.3831& Y= 0.0478x +0.1537 respectively. (Table 2, Fig. 1)

Table 2: LC_{50} value and its regression equation of antilarval and antipupal activity of aqueous and ethanolic extract of *Clerodendrum phlomidis* against *Anopheles stephensi.*

Sr. No.	Activity	Plant extract	LC_{50} (mg/l)	LC_{90} (mg/l)	Regression Equation	R^2
1	Antilarval activity	Aqueous extract	150.32	250.32	y = 0.400x - 10.13	R^2 = 0.904
2	Antilarval activity	Ethanolic extract	90.39	157.28	y = 0.598x - 4.057	R^2 = 0.972
3	Antipupal activity	Aqueous extract	139.95	231.07	y = 0.439x - 11.44	R^2 = 0.906
4	Antipupal activity	Ethanolic extract	121.30	204.29	y = 0.482x - 8.471	R^2 = 0.952

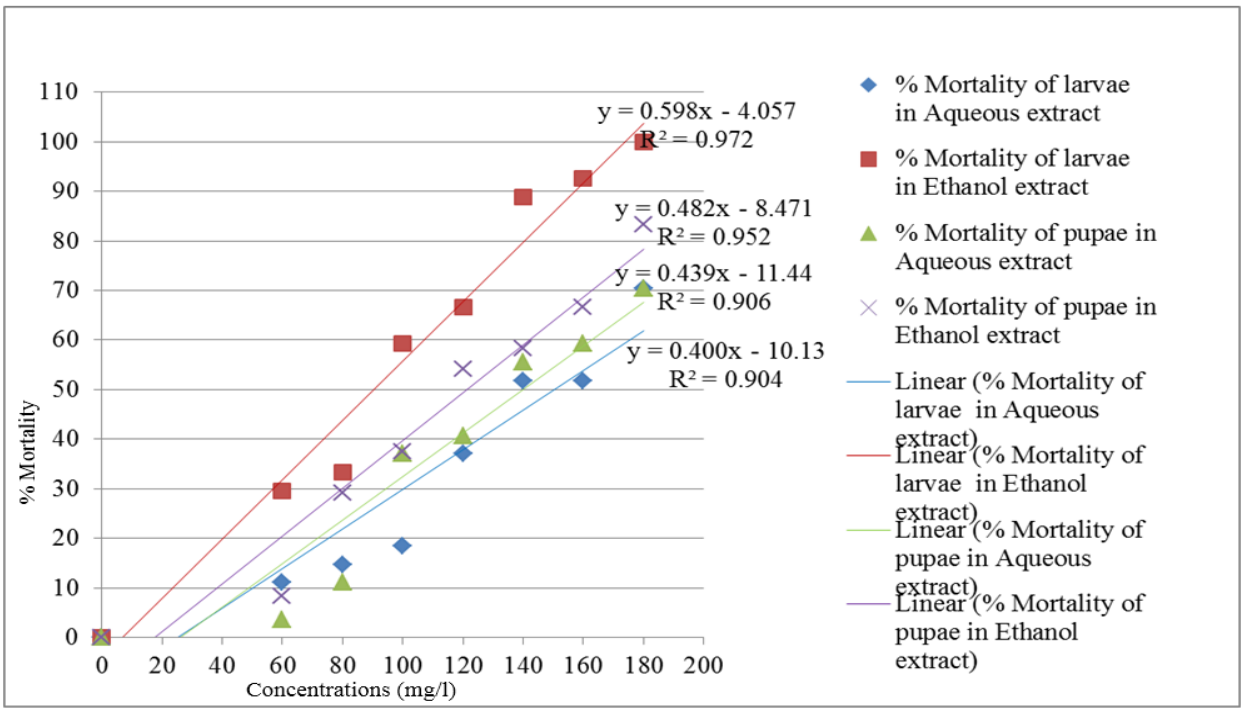

Fig 1: % Mortality of larvae and pupae of *Anopheles stephensi* in aqueous and ethanolic extract of *Clerodendrum phlomidis.*

4. Discussion

As prevention is better than cure, the best way to control vector-borne diseases is to control the population of vectors. So there forth many researchers [2, 6, 15, 17, 18, 28, 29] have been conducted experiments on the mosquitoes for antilarval and antipupal activity by using plant derived chemicals. These are non-toxic to humans, domestic animals and serve as useful basis for the development of eco-friendly, cost effective and precise insecticide [17]. In the present study *Clerodendrum phlomidis* leaf extracts tested against *Anopheles stephensi* for the antilarval and antipupal activity. Among the different plant parts used for the preparation of medicine, the leaves were found to be the most frequently used plant parts in the preparation of majorities of the remedy [30]. The ethanolic extract showed higher larval and pupal mortality compared to aqueous extract. The leaf extract of *Clerodendrum phlomidis* were compared with other plant extracts and found positive antilarval and antipupal activity. The medicinal plant *Adansonia digitata* proved to be antilarval against *Anopheles stephensi* as LC_{50} and LC_{90} values of methanol extract were 78.18 mg/l & 155.42 mg/l, chloroform extract were 88.55 &168.14 mg/l, benzene extract were 97.13 &176.16 mg/l respectively [31]. The LC_{50} and LC_{90} values of hexane extract of *Momordica charantia* against *Anopheles stephensi* were 66.05 & 125.96 respectively [32]. The petroleum ether and methanol extract of cactus plant *Agave sisalana* has LC_{50} & LC_{90} values 77, 221 mg/l and 586, 109 mg/l respectively [10]. In the antilarval study against *Anopheles subpictus*, LC_{50} and LC_{90} values of bark methanol extract of *Annona squamosa*, leaf ethyl acetate extract of *Chrysanthemum indicum* L. and leaf acetone extract of *Tridax procumbens* L. were 93.80, 39.98, 51.57 mg/l and 524.90, 145.70, 226.56 mg/l respectively [27]. Results obtained after the treatment of leaf extract of *Clerodendrum phlomidis* against *Anopheles stephensi* were encouraging. The larval and pupal activity may be due to the active compounds present in *Clerodendrum phlomidis* like steroids such as *β*-sitosterol, clerosterol, campesterol, 4*α*-methylsterol, or terpenes like monoterpenes, diterpenes, triterpenes, or α-amyrin, *β*-amyrin, and flavonoids cynaroside, kaempferol, apigenin, luteolin, [21, 23, 30]. The further study with the specific phytochemicals may show higher larvicidal and pupaecidal activity. The plant extract are easy to handle, inexpensive and safe natural products for mosquito control. Though several compounds of plant origin have been reported as larvicides, there is a wide scope for the discovery of more effective plant products. Further research may lead to improved formulations with enhanced activity to replace the conventional hazardous insecticides for mosquito control.

5. Conclusion

The larvae and pupae of malarial vector *Anopheles stephensi* were found to be susceptible against the ethanolic and aqueous leaf extract of *Clerodendrum phlomidis.* The ethanolic leaf extract were more promising showing 100% mortality at 200 mg/l against larvae as well as pupae of *Anopheles stephensi* as compared to 84.44% and 81.44% mortality of aqueous leaf extract of *Clerodendrum phlomidis* respectively. The increased amount of solubility of steroids, terpenes and flavonoids in

ethanol than water may be the reason of higher activity. *Clerodendrum phlomidis* ascertained to be potential herbal anti-mosquito agent and further study will show promising stratagem for mosquito control.

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