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Full Length Research Article

Developmental Effects of Acetone, Hexane and Chloroform Leaf Extracts of *Millingtonia hortensis* L. f. (Bignoniaceae) against *Aedes aegypti* (Diptera: Culicidae)

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Abstract

Aedes aegypti are responsible for transmitting various life-threatening diseases all over the world and created a burden of society. Excessive use of synthetic insecticides, with a complete lack of awareness of the strategy of changing the pesticides, led to resistance to pesticides along with environmental pollution and health risks to humans and non-target biota. Thus, insecticide resistance in *A. aegypti* poses a significant threat to public health worldwide. Among the alternatives to synthetic insecticides for the control of these vectors, the use of natural plant products deserves attention. The use of plant-based insecticides that are less harmful to the environment and without known resistance development. Qualitative phytochemical analyses of the plant extracts were (acetone, hexane, chloroform leaf extracts of *M. hortensis* carried out using the standard protocol. The acetone, hexane, chloroform leaf extracts of *M. hortensis* inhibited normal growth and development of *A. aegypti* larvae, pupae and prolonging larval, pupae duration when compared to control. The acetone, hexane, chloroform leaf extracts of *M. hortensis* can be used as an effective bio insecticide to control mosquitoes as environment friendly solution.

***Key words*:** *A. aegypti,* Larval and pupal period prolongation, Acetone, Hexane, Chloroform leaf extracts of *M. hortensis*

Mosquitoes are the most important pest, cause several infectious diseases e.g., dengue, chikungunya, yellow fever, zika, malaria, filariasis. Mosquito borne diseases are prevalent in more than 100 countries across the world. Dengue fever is regarded globally as the most important arthropod-borne viral diseases caused by *A. aegypti*. It rests in cool shaded places in house such as wardrobe, under furniture and laundry areas. About 50% of the world's population lives in areas where there is a risk of dengue transmission [1]. It is endemic in at least 100 countries in Asia, the Pacific, the Americas, the Africa and the Caribbean. Based on the data collected by National Vector Borne Disease Control Programme in India, dengue fever was first accounted in 1956 from Vellore District in Tamil Nadu and the first dengue haemorrhagic fever outburst was informed from the eastern coast in 1963 [2]. According to the NVBDCP (National Vector Borne Disease Control Programme) number of dengue and chikungunya cases 2022 in India was 1,10, 473 and 86 deaths and in Tamil Nadu was 4771 and 4 deaths; in India was 5320 and no deaths and in Tamil Nadu was 149 and no deaths respectively. The prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. Synthetic insecticides remain the first line of defence against *A. aegypti*. However, starting in the 1950s, resistant strains of the insect began to be identified. The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds, however, one major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment [3].

Thus, the Environment Protection Act in 1969 has framed a number of rules and regulations to check the application of chemical control agents in nature [4]. These factors have resulted in an urge to look for environment friendly, cost-effective, biodegradable and target specific insecticides against mosquito species. One of the most effective alternative approaches under the biological control programme is to explore the floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple and sustainable method of mosquito control. Thus, there is very little chance of pests developing resistance to such substances [5]. Phytochemicals are naturally occurring insecticides obtained from plants. They are biodegradable, have no ill effects on non-target organisms and do not have the disadvantages associated

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with injudicious application of synthetic insecticides [6-7]. In this sense, substances extracted form plants present a great perspective for the control of *A. aegypti*. Some of the plant leaves extracts are tested for their diverse insecticidal properties on the medically important mosquitoes: ethanolic extract of *Cymbopogon citratus, Ricinus communis*. *Allium sativum* [8]; crude extract of *Phyllanthus acidus* [9]; methanol, acetone, hexane, aqueous extract of *Thuja orientalis* [10]; aqueous extract of *Pangium edule* [11]; ethyl acetate extract of *Plectranthus amboinicus, Sphagneticola calendulacea* [12]. As far as our literature survey that there was no information available on the developmental effects of acetone, hexane, chloroform leaf extracts of *M. hortensis* against *A. aegypti.*

Millingtonia hortensis L.f is an important medicinal plant in Southern Asia ranging from India, Burma, Thailand and South China. It is commonly known as Cork tree, Tree Jasmine and Maramalli in Tamil and the sole species in the genus *Millingtonia*. The tree grows to height of between 18 to 25 metres and has a spread of 7 to 11 meters. The leaf is imparipinnate and resembles that of the neem. The white flowers come as large panicles which emit a pleasant fragrance. It is an ornamental tree with pleasant flowers, which make suitable as a garden tree. The leaves are used a substitute for tobacco in cigarettes [13]. The leaves of *M.hortensis* are used as antipyretic [13]; antiasthmatic [14]; tonic in folklore medicine, antibacterial, [15]; larvicidal [16]. The aim of the present study is:

- Qualitative phytochemical analysis of acetone, hexane, chloroform leaf extracts of *M. hortensis.*
- Prolongation of developmental time (larvae and pupae) of *A. aegypti* with effect of acetone, hexane, chloroform leaf extracts of *M. hortensis.*

MATERIALS AND METHODS

The eggs of *A. aegypti* were collected from National Institute for Communicable Disease (NICD), Mettupalayam, Coimbatore (Dt), Tamil Nadu, India. They were hatched, reared and have been still maintained for many generations in the laboratory. The larvae were reared in plastic cups (27±2ºC, relative humidity at 70-80%) and 12:12h light and dark cycles provided with commercial fish food *ad libitum* until their metamorphosis to pupal stage [17]. The pupae were collected from culture trays and were transferred to glass beakers. The pupae containing glass beaker were kept inside mosquito cage for adult emergence. The adult female *Ae. aegypti* were fed by human arm [18-19]. Both females and males were provided with 10% glucose solution on cotton wicks [20]. A plastic cup (200 ml) (ovitraps) lined with filter paper containing water was kept in the cage to collect the eggs.

Collection and preparation of plant extracts

M. hortensis leaves were collected from Government Arts College campus, Coimbatore, Tamil Nadu, Southern India. The identification of the plant was authentified at BSI Coimbatore (NO: BSI/SRC/5/23/2015/Tech/2168). The leaves washed with distilled water and then they kept for drying under shade at room temperature (27± 2ºC) for about 2 weeks till they dried completely. The dried leaves were finely powdered using electric grinder. Powdered plant material (100g) was soaked in acetone, hexane, chloroform (1000 ml) in airtight wide mouth bottle and kept separately for 4 days with periodic shaking. After that, the extracts were filtered using Whatman No.1filter paper and kept in Petri dishes for drying at room temperature [21]. Dried extracts where then used for the preparation of stock solution. This stock solution was used to prepare the desired concentrations of the extract for exposure of the mosquito larvae.

Qualitative phytochemical analysis of acetone, hexane, chloroform leaf extracts of M. hortensis

Qualitative phytochemical analyses of the plant extracts were carried out using the standard protocol [22-23].

Laboratory assay for larval and pupal duration

To determine the effect of acetone, hexane, chloroform leaf extracts of *M. hortensis* on the length (duration) of the larval stage (larva-pupation) of different concentrations test solutions were prepared. Fifty first instar larva was placed in the extracts and allowed to develop further. The medium was watched every 24 hours. The total larval duration in days was recorded from I-instar to pupation. In parallel, the duration of larval stage for the larvae reared in control was calculated for comparison [24]. The data were statistically examined using Student's *t*-test.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis of acetone, hexane, chloroform leaf extracts of M. hortensis

Qualitative phytochemical analysis revealed the presence of different phytochemicals such as carbohydrates, terpenoids, coumarins and steroids (acetone); carbohydrates, tannins, flavonoids, triterpenoids, phenols, coumarins, steroids and phytosteroids (hexane); tannins, flavonoids, alkaloids, quinones, terpenoids and steroids (chloroform) (Table 1).

Table 1 Qualitative phytochemical analysis of *M. hortensis* acetone, hexane, chloroform leaf extracts

Phytochemical constituents	Acetone	Hexane	Chloroform
Carbohydrates	+	+	-
Tannins	-	+	+
Saponins	-	-	-
Flavonoids	-	+	+
Alkaloids	-	-	+
Quinones	-	-	+
Glycosides	-	-	-
Cardiac glycosides	-	-	-
Terpenoids	+	-	+
Triterpenoids	-	+	-
Phenols	-	+	-
Coumarins	+	+	-
Steroids	+	+	+
Phytosteroids	-	+	-
Phlobatannins	-	-	-
Anthraquinones	-	-	-

(+): Present; (-): Absent

Effect of acetone, hexane, chloroform leaf extracts of M. hortensis on total larval duration of A. aegypti

The different solvent leaf extracts of *M. hortensis* at 0.1, 0.3, 0.7, 0.9, and 0.011% (acetone); 0.1, 0.3, 0.7, 0.9, and 0.011% (hexane); 0.1, 0.3, 0.7, 0.9, and 0.011% (chloroform); tested against the *A. aegypti* was found to prolong larval and pupal period. In the control it took 9 days for all the larvae to become pupae, whereas the acetone took 11, 17, 19, 24, and 30 days; hexane took 11,14,16,21 and 26 days; chloroform took 11, 17, 18, 26 and 29 days; with reference to hierarchy of concentrations (Table 2).

Table 2 Prolongation of larval duration of *A. aegypti* reared in the medium of different concentrations of acetone, hexane, chloroform leaf extracts of *M. hortensis*

Concentrations (%)	Larval duration (Days)				Total larval and pupal duration (Days)
	I- II	II - III	III - IV	IV- Pupa	
Control	2	2	2	3	9* ± 0.001
0.1	2 (a)	3(a)	2(a)	4(a)	11*
	2(h)	3(h)	2(h)	4(h)	11*
	2(c)	3(c)	2(c)	4(c)	11* ± 0.003
0.3	2(a)	3(a)	3(a)	6(a)	17*
	2(h)	3(h)	2(h)	7(h)	14*
	2(c)	3(c)	3(c)	6(c)	17* ± 0.001
0.7	2(a)	4(a)	4(a)	9(a)	19*
	2(h)	3(h)	3(h)	8(h)	16*
	2(c)	4(c)	4(c)	8(c)	18* ± 0.003
0.9	2(a)	5(a)	7(a)	10(a)	24*
	2(h)	3(h)	4(h)	12(h)	21*
	2(c)	6(c)	6(c)	12(c)	26* ± 0.005
0.011	2(a)	6(a)	10(a)	12(a)	30*
	2(h)	4(h)	6(h)	14(h)	26*
	2(c)	7(c)	7(c)	14(c)	29* ± 0.003

Mean (± SD) of 3 replicates
*Significantly different from control, P < 0.001
a- acetone leaf extract
h- hexane leaf extract
c- chloroform leaf extract

The control of mosquito by chemical substance is not safe at present of insecticide resistance by vectors and environmental imbalance. The extract which is obtained from plant parts like leaves, roots, flower, bark, seed and fruit in their crude extracts has been used as conventional insecticides [25]. The secondary compounds of plants are vast repository of compounds with a wide range of biological activities [26]. Earlier several researchers have shown that plants extracts treated against developmental stages of mosquitoes lead to prolongation of larval and pupal developmental period.

One of the significant observations made in the all the experiments was the prolongation of the larval developmental period for 4 to 7 days in the chloroform, petroleum ether, hexane extracts of *Clerodendron inereme* treated third and fourth instar larvae *A. aegypti* compared to the control groups where the third instar larval period was found to be 2 days and fourth instar was for 3 days [27]; larval duration increased in treated individuals and total development period (larval and pupal development) took twelve, thirteen and thirteen days in *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* whereas in control it was ten days [28]; results revealed that treated individuals took prolonged larval period significantly when compared to control with acetone leaf extracts of *Artemisia herbalba, Lavandula multifida, Peganum harmala*. The pupal duration significantly increased in treated individuals only with hexane extract of *Lavandula multifida*. The larval period lasted 7 to 7.7 (control 6 days) and pupal period lasted 2.33 to 3 days (control 2 days) in treated individuals. Total developmental period (larval and pupal development) took 8 to 10 days (control 8 days) in treated individuals while, significantly increased with acetone leaf extract of *Lavandula multifida* [29]; the crude alkaloidal extract from leaves of *Ricinus cummunis* and *Argemone mexicana* were evaluated for growth inhibitory activity against *A. aegypti* mosquito larvae. The extracts of both the plants demonstrated promising prolonged larval and pupal period with an overall increase in the developmental period of mosquito life cycle [30]; treatment of Ist instar of *C. quinquefasciatus* with 114 ppm of methanol extract of *Blumea mollis* shows an extension of developmental period up to 23.5 days; methanol extract of *Vernonia cinerea* shows an extension of developmental period up to 24 days than that of control 12 days. A maximum of extended developmental period of 25.5 days was observed when the first instar of *C. quinquefasciatus* was treated with 85 ppm of the methanol extract of *Centratherum punctatum* [31]. The phenomenon of prolongation of the larval development period suggests that the acetone, hexane, chloroform leaf extracts of *M. hortensis* interfered with development process leading to survival of the larvae without moulting and eventually leading to death at higher concentrations. In the present study, lengthening of larval and pupal periods indicates the interference of the bio–active compounds of acetone, hexane, chloroform leaf extracts of *M. hortensis* with the normal hormonal activity coordination of the metabolic process of the developing stages. Prolongation of development period of mosquito larvae treated with plant extracts were generally attributed to interference of the active ingredients of the extracts with the endocrine system [32].

CONCLUSION

This study demonstrated the development potential of the acetone, hexane, chloroform leaf extracts of *M. hortensis* against the larvae and pupae of *Aedes aegypti*. The results showed that *M. hortensis* extracts has a developmental effect against the *Aedes aegypti*. This study is significant to develop eco-friendly insecticides from *M. hortensis* to curb mosquito larvae and pupae which can provide an alternate to synthetic insecticides.

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