Phytochemical screening and Antivectoral activity of Muntingia calabura

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Abstract

Mosquitoes are the vectors of many severe diseases like malaria, dengue, yellow fever, filariasis, chikunguniya, west nile virus, western equine encephalitis and considered as one of the life threatening creatures in the world. The present study aims to identify the phytochemical compounds and larvicidal activities of the two extracts, ethanol and chloroform, present in the leaves of *Muntingia calabura* against the filarial vector *Culex quinquefasiatus* and to compare the efficiency of these two extracts. The plant leaves were collected, dried and powdered and the extracts were prepared using chloroform and ethanol and then series of tests were conducted. The eggs were collected, hatched and reared in the laboratory. The colony of larval instars were kept in glass beaker along with dechlorinated water and added with desired concentrations of plant extract and the mortality rate were calculated. The mortality of larvae was observed at 48hrs. The LC_{50} and LC_{90} values of *M.calabura* leaf extracts were depicted and for the each test the concentration of the extract is increased. It was observed that the mortality increases as the concentration of extract increases. Among the two extracts, *M. calabura* ethanol leaf extract was found to be more effective than the chloroform extract against the filarial vector *Culex quinquefasiatus* which is cost efficient and ideal ecofriendly approach for the control of mosquitoes.

Keywords: Phytochemical, Antivectoral, Muntingia calabura, *Culex quinquefasiatus,* Chikunguniya.

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1. Introduction

Mosquitoes act as a vector for many diseases like malaria, dengue, chikungunya, west nile virus, and yellow fever, filarious. Among these dengue is one of the wide spreading disease by mosquito. Virus belongs to the Family Flaviridae spreads dengue in urban and semi-urban areas. Dengue fever considered as an infectious disease due to increased level of trade, travel, lack of sanitation in effective mosquito control and urbanization. This is endemic in most of the countries. So far no effective drug or vaccines found. For dengue fever only remedies are followed to control the disease carrying mosquito.

The most imporatant mosquito species *Culex quinquefasciatusis* is the main vector of filarial parasite Wuchereriabancrofti and a variety of arboviruses on the East African coast and the island of indian ocean [1]. This *Culex* is found mainly in rainy season, diches, fish pond, rice field. They serve as a suitable place for breeding mosquitoes[2]. *Culex* species becoming resistant to chemical pesticide and a transmit zoonotic diseases that affect human and other animals [3]. The chemicals obtained from plants have exploded as weapon in future mosquito control program.[4]. Different chemical group of phytochemicals such as steroids, alkaloids, terpenes and phenolic compound were found for insect control. The management ability of insects whereas with age of the plant, species, parts extracted, collection site and solvent used for extraction[5]. Natural products are generally preferred in vector control measure due to their less deletorious effect thus identifying active compounds from natural products against mosquitoes is worthwhile [6]

Muntingia calabura can reach upto 40 feet found in tropical region worldwide. It can growth in poor soil but do no grow in saline condition [7]. The vernacular name of *Muntigia calabura* in tamil is "Sakkaraipalam maram". The leaves of the plant are alternate and covered with short hair. The fruit is small and edible which is have an unique savour which gratifies human tatse. The plant absorbs less water. It contains antioxidants, sinamic acid, flavonoids, tocopherols and coumarin and the plant extract possess antimicrobial properties [8].It has a significant role in therapeutic area. The flowers of this plant also used as an source of paper pulp in Brazil[9]. *M.calabura* plant also possess anti diabetic activity, relives pain, cold and headache, antibacterial and cytotoxic activity [10]. The potential ethanol extract of M.calabura possess anti diabetic activity[11]. Antioxidants are present in the leaves of this plant protect our body from many diseases. M.calabura has antiseptic, an anti-inflammatory activity and are widely used in the treatment of cancer[12]. This plant is used as an insecticide to control larvae and pupa of mosquitoes [13].

2. Materials and Methods

2.1 Collection of eggs

The eggs of *Culex quinquefasciatus* were collected from NCID, in and around Coimbatore with the help of 'O' type brush. The eggs were transferred to 18×13 ×4 cm sized enamel trays which contains 500 ml water in the laboratory. The eggs were maintained and the hatched larvae were reared for many generations.

2.2 Maintenance of mosquito larvae

In the laboratory, the freshly hatched larvae were fed with dog biscuit and yeast in the ratio of 3:1 till it reaches the pupal stage, where the larvae were reared in the plastic cups. Water have been changed regularly and the dead ones are removed at that sight. The larval culture and the breeding cups used in the experiment for the study were covered with muslin cloth to prevent the contamination from the external environment.

2.3 Preparation of plant extract

The leaves of *Muntingia calabura* were collected from in and around Coimbatore and brought to the laboratory. 250 g of fresh plant leaves were washed with distilled water and placed to dry under the shade. The dried leaves were then powdered and sieved. The leaf powder were packed for solvent extraction in a Soxhlet apparatus (Precision scientific co., Coimbatore)[14]. The extracts were prepared using chloroform and ethanol solvents (Modern Scientific Company, Coimbatore. 99% purity). 72 hrs were taken for the extraction and the temperature varies according to the solvent. The dried residues were obtained by letting the yield extract of 50 g to get evaporated.

2.4 Phytochemical screening

The ethanol is high polar and choloroform is non polar were analysed for the phytochemical compounds. The plants of *M.calabura* contains bioactive compounds. The phytochemical screening were carried out using standard procedure to detect the presence of bioactive compounds like flavanoids, terpenoids, alkaloids, saponin, anthroquinones, quinones, phenols, glycosides.

2.5 Larval toxicity test:

Culex quinquefasciatus larvae were used for the larvicidal activity. Totally 25 members of larvae of I,II,III,IV instar were kept in 500 ml glass beaker and it contains 249 ml of dechlorinated water and 1 ml of concentration of extract was added with same concentration[15]. As the rate of concentration increases the mortality rate also increases. Larval food were given for the test larvae. Five trials were made at each tested concentrations and the mortality of larvae was observed at 48hrs and for the each test the concentration of the extract is increased the mortality rate was recorded. The Lethal concentration $LC_{50,}$ LC_{90} were calculated from toxicity data using probit analysis [16]

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

$$\text{Percentage mortality } = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

The control was done by mixing 1 ml of acetone with 249 de-chlorinated water. Using Abbott's formula, the control mortalities were corrected [17].

3. Results

The larvicidal activity of *Muntingia calabura* leaves extracts of ethanol and chloroform at various concentrations against the filarial vector *Culex quinquefasciatus* were observed. The mortality rate increases as the concentration of the extract increases.Both chloroform and ethanol extract showed the larval mortality rate[18]. The percentage mortality larvae of *Culex quinquifasciatus* where treated with various concentration of the leaf extract reported for LC_{50} and LC_{90} [19]. The LC_{50} and LC_{90} values of *M.calabura* leaf extracts were depicted for 48hrs. The LC_{50} values were 400ppm, 200ppm, 100ppm, 50ppm and 25ppm provides the result for ethanol extract of *Muntingia calabura* against the fourth instar larvae of *Culex quinquefasciatus* which showed 86% larval mortality at 48hrs at 400ppm concentration (Table 1). Similarly the chloroform extract against the *Culex quinquefasciatus* showed 85% mortality rate at 48 hrs at 400 ppm concentration(Table 2)[20]. It was evident that the extract was effective against the larvae of mosquitoes. As the concentration increases I and II instars mortality reaches 100% and III, IV instars concentration increases, the mortality rate reaches 86%.

By comparing the efficiency of these two extracts, ethanol extract showed the highest mortality rate than the chloroform extract. The mortality rate also increased when the concentration of the extract is increased. This showed the antivectoral activity of the plant extract against the *Culex quinquefasciatus.*

Phytochemical screening of the ethanol and chloroform extract revealed the presence of bioactive compounds in these two extract. The ethanol extract contains quinones, steroids, saponin, flavanoids, terpenoids, alkaloids and phenols. Glycosides is absent in *M.calabura* ethanolic and chloroform extract[21]. The chloroform extract contains flavonoids, saponins, quinone and tannins (Table 3).

Table 1: Larvicidal activity of ethanol extract of *M.calabura* against *Culex quinquefasciatus* at 48 hours

Ethanol extract concentration (ppm)	Larval Motility (%)	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	Chi square value
25	38	80.6 (38.9 – 114.5)	445.8 (374.0 – 565.5)	1.5
50	49			
100	52			
200	68			
400	86			

Table 2: Larvicidal activity of chloroform extract of *M.calabura* against *Culex quinquefasciatus* at 48 hours

Chloroform extract concentration (ppm)	Larval Motility (%)	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	Chi square value
25	34	117.7 (83.7 – 149.7)	465.2 (394.0 – 579.9)	0.4
50	38			
100	45			
200	61			
400	85			

Table 3: Phytochemical compounds of ethanol and chloroform extract of *M.calabura* leaves

Compounds	Solvent used	Presence or absence
Phenols	Ethanol, Chloroform	Presence
Terpenoids	Ethanol	Presence
Flavanoids	Ethanol, Chloroform,	Presence
Glycosides	Ethanol	Absence
Quinones	Ethanol, Chloroform	Presence
Steroids	Ethanol	Presence
Saponin	Ethanol, Chloroform	Presence
Alkaloids	Ethanol	Presence

4. Discussion

Mosquitoes cause various diseases in human. Mosquitoes normally breed in water and applying synthetic pesticides are harmful for the environment [22]. The plant extract has the potency to inhibit their populations. The plant compounds perform effectively against the mosquito vectors. Many plant extract possess larvicidal activity against wheareas mosquito species. It was pointed out that most promising botanical mosquito control agents of selected plant families with medicinal properties namely *Asteracae, Meliaceae* and *Rutaceae* [23]. This is one of the alternative method in mosquito control and less hazardous to humans. The larvicidal activity of petroleum ether, acetate, Chloroform, ethyl acetate and methanol extracts of *J.adhatoda* leaf against *Culex quinquefasciatus* larvae indicated the highest mortality rate compared to other solvents [24].

The details of Larvicidal activities of ethanol and chloroform extracts against *culex quinquefasciatus* were reported in the present study. The high mortality rate was observed in ethanol extract. The preliminary phytochemical analysis of *M.calabura* was found to have the presence of flavanoids, tannin, phenols, saponin, alkaloids, terpenoids. These phytochemicals have the bioactive compounds that effectively kills the filarial vector of mosquitoes.

In the present study the phytochemical compounds of the plant extract acts as larvicide which kills the larvae of the mosquitoes due to the presence of effective bioactive compounds. To create awareness among the society this plant has been taken for our study. This plant possess good larvicidal activity against the mosquito vector. The phytochemicals like phenols, ethanol, flavanoids, terpenoids are present in the ethanol extract and the ethanol extract was found to be more effective against the filarial vector. By comparing the efficiency of these two extracts ethanol extract showed the highest mortality rate. The extract of ethanol exerted good mortality rate at 400ppm concentration than chloroform extract. Instead of using pesticides these extracts can be used. Moreover these results could be useful in the research for selecting more efficient, newer, more selective biodegradable, comparatively less hazardous pesticide to the environment. This is one of the eco friendly bio pesticide used for the control of mosquito vectors[25]. The larvicidal activity of *Muntingia calabura* extract is more effective against the mosquito vectors. This research showed the usage of plant extracts in fields to prevent the bioaccumulation and ecological damage by avoiding the use of synthetic pesticides.

5. Conclusion

The leaf extract of *Muntingia calabura* was observed to have good larvicidal activity against the filarial vector *Culex quinquifasciatus.*This is the first study to report the larvicidal activity of the plant *Muntingia calabura* against the mosquitoes. The mortality rate of these two extracts were studied. As the concentration of the extract increases the mortality rate also increases. Both chloroform and ethanol extract showed the mortality rate against the larvae of mosquitoes. By comparing the efficiency of these two extracts, ethanol extract was found to be more effective against the mosquitoes and showed the highest mortality rate than the chloroform extract. Thus the plant leaves of *Muntingia calabura* contain essential bioactive compounds to control mosquitoes in environment friendly manner

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