



ISSN: 0976-3031

Available Online at <http://www.recentscientific.com>

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research  
Vol. 9, Issue, 5(1), pp. 27113-27119, May, 2018

**International Journal of  
Recent Scientific  
Research**

DOI: 10.24327/IJRSR

## Research Article

# PHYTOCHEMICAL ANALYSIS AND LARVICIDAL ACTIVITY OF AQUEOUS AND ETHANOL EXTRACT OF SELECTIVE MEDICINAL PLANTS AGAINST MALARIA AND DENGUE VECTOR

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DOI: <http://dx.doi.org/10.24327/ijrsr.2018.0905.2190>

### ARTICLE INFO

#### Article History:

Received 24<sup>th</sup> February, 2018  
Received in revised form 19<sup>th</sup>  
March, 2018  
Accepted 16<sup>th</sup> April, 2018  
Published online 28<sup>th</sup> May, 2018

#### Key Words:

Bioactive compounds, dengue and malarial vector and plant extracts

### ABSTRACT

Bioactive compounds present in the medicinal plants which are anti larvicidal activity against dengue and malarial vector. Phytochemicals, which are in two categories - primary and secondary. Medicinal plants have anti-fungal, anti-bacterial and anti-inflammatory. The present study involves phytochemical analysis of selective plants. The leaf of plants such as *Carica papaya*, *Azadirachta indica*, *Ocimum sanctum*, *Cymbopogon citratus*, *Mentha spicata*, *Aloe vera*, *Vitex negundo*, *Plectranthus amboinicus*, *Senna auriculata* and *Manilkara zapota*, bulb of *Allium sativum* and flower of *Targetes erecta* was washed, air dried aqueous and ethanol extract of leaf sample were used for the phytochemical analysis to find out the phytochemical constituents in the plant such as alkaloid, glycoside. Phytosterol, flavonoid, phenol, protein and carbohydrate. Also analysis the larvicidal activity of medicinal plants in larvae of *Aedes* and *Anopheles* in aqueous and ethanol extracts and recorded the mortality rate in 24 and 48 hours.

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## INTRODUCTION

The dengue fever is one of the life threatening diseases caused by dengue virus (*Flavivirus*) that is borne and transmitted by mosquitoes living in tropical and subtropical climates worldwide, mostly in urban and semi-urban areas. As per the estimates of World Health Organization, every year, 50 million people across the world are infected by dengue and about 2/5 of the world population (2.5 billion people) are at risk from this dreadful disease, which spreads over about 100 countries. Malaria is a major parasitic disease in the world, especially in Africa. It is responsible for 500 million new cases and 2 to 3 million deaths every year. *Plasmodium falciparum* the most widespread etiological agent for malaria. (WHO, 2008).

Dengue and malarial fever causes mortality and morbidity around the world. Medicinal plants have been used widely to treat a variety of vector ailments such as dengue and malaria. The demand for plant-based medicines is growing as they are generally considered to be safer, non-toxic and less harmful than synthetic drugs. Ethnobotanical surveys and laboratory investigations are needed established the potential of identified species in contributing to dengue and malaria control (Bandeira *et.al.*, 2001 and Bower *et.al.*, 1995).

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical

constituents. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds. (Nostro *et. al.*, 2000 and Kamaraj and Rahumen 2011).

Due to the pathogenic diseases and serious harms caused by mosquitoes, controlling them has been the primary subject of several new researches over the past few years. Current research trends use plant extracts as alternative larvicides because they contain various phytochemicals that are specific in killing mosquito larvae without harming other organisms and the environment. Instead of using synthetic larvicides, the use of these plant-derived products in controlling mosquito larvae is inexpensive and environment-friendly (Invest and Lucas, 2008). Hence the objective of the present study was to evaluate the larvicidal activity of plant extracts (Lima *et.al.*, 2011).

## MATERIALS AND METHODS

### Study area

We collected the plants from Tiruchirappalli and its surroundings. It is approximately located at 10.7905° N,

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78.7047° E and its land mass is 16.72 Km<sup>2</sup> and its population is 11,29,422

### **Medicinal Plants Used For Phytochemical and Larvidial Activity**

*Carica papaya*, *Azadirachta indica*, *Ocimum sanctum*, *Cymbopogon citratus*, *Mentha spicata*, *Aloe vera*, *Vitex negundo*, *Plectranthus amboinicus*, *Senna auriculata*, *Manilkara zapota*, *Allium sativum* and *Targetes erecta*.

### **Collection of plant material**

The leaves of *Carica papaya*, *Azadirachta indica*, *Ocimum sanctum*, *Cymbopogon citratus*, *Mentha spicata*, *Aloe vera*, *Vitex negundo*, *Plectranthus amboinicus*, *Senna auriculata*, *Manilkara zapota*, bulb of *Allium sativum* and flower of *Targetes erecta* were collected washed thoroughly blotted and shade dried

### **Extraction**

About 10 gm of dry sample of each plant was macerated with sterile water, Acetone and Ethyl alcohol and left to stand at room temperature for 48hrs. Then mixture was filtered through a Whatman no.1 filter paper by suction. Filtrate was evaporated under vacuum for 40°C unit.

### **Collection of Mosquito Larvae**

Mosquito larvae were gathered from the water present in the exposed coconut shells in Ramalingam Nagar, Tiruchirappalli. Mosquito larvae were brought to a small room enclosed with a mosquito net and then transferred to a molder with clear tap water. The larvae were kept in a small room which is enclosed with a mosquito net for safety precautions in case of the fast emergence of the larvae into an adult mosquito.

**Phytochemical screening:** preliminary qualitative Phytochemical examinations were carried out to identified the secondary metabolites present in the various aqueous, acetone and ethanol extract of leaf, bulb and flower all the extracts as per the Harborne (1998) methods.

### **Types of Mosquito Taken For Study**

*Anopheles* and *Aedes aegypti*

### **Mosquito Larvae**

Larvae of a mosquito can be identified from any other aquatic insects since it has a combination of two characters, they have no legs and the thorax is wider than the head or abdomen. The three divisions of the body part mosquito larvae are head, thorax and abdomen. The structure of three body regions serves as the basis for identifying the mosquito larvae. The mosquito larva was identified using a compound microscope. A small amount of water with a mosquito larvae was drop in a slide to be able to view the specimen in the compound microscope. The target mosquito larva in this study was the third instar larva of dengue carrying mosquito *Aedes aegypti*. And *Anopheles* (Mohan *et.al.*, 2006 and Rajkumar and Jabanesan 2009)

*Aedes aegypti* larvae can be distinguished from any other mosquito larvae since it normally has a single hair, a three branch hair tufts on each side of the air tube. When the hair tuft has two or more branches all branches arise from the same socket. Other species have two or more hairs, branches and hair

tufts on each side of the air tube or siphon. Identified *Aedes aegypti* mosquito larvae (Harborne, 1998).

The mosquito larva has a well-developed head with mouth brushes used for feeding, a large thorax and a nine-segment abdomen. It has no legs. In contrast to other mosquitoes, the *Anopheles* larva lacks a respiratory siphon, so it positions itself so that its body is parallel to the surface of the water. Both the larvae were separated from the other mosquito species and were placed in a water- filled plastic molder.

### **Mosquito Larvicidal Bioassay**

The efficacy of the plant extracts as larvicide against the dengue-vector *Aedes aegypti* mosquito and *Anopheles* was evaluated in accordance with the guidelines of World Health Organization 13. Batches of 10 third-instars larvae of *Aedes aegypti* and *Anopheles* were placed separately in a small plastic container with 50 ml dechlorinated water and lay in the netted area in the Laboratory room at 30-32°C. For the control group, the mosquito larvae were exposed to 60 mg/mL water since it is the solvent used in the extraction of different plant samples. The experimental group is the Aquous, acetone and Ethyl alcohol extracts of the flower, bulb and leaf of *carica papaya*, *Azadirachta indica*, *Ocimum sanctum*, *Allium sativum*, *Cymbopogon citratus*, *Mentha spicata*, *Aloe vera*, *Vitex negundo*, *Senna auriculata*, *Plectranthus amboinicus*, *Targetes erecta* and *Manilkara zapota* with 10 mg/mL, 20 mg/mL, 50 mg/mL concentrations. These concentrations were chosen after the pre-test/pretreatment conducted. Identification of the mosquito larvae were done by tapping it with a needle in the siphon or cervical area. Each treatment was conducted in three replicates. The effects of the plant extracts were monitored through carefully counting s number of dead larvae after 24 and 48 hours of treatment, and the percentage mortality was computed( WHO, 2015).

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

## **RESULTS AND DISCUSSION**

Table-1, shows the phytochemicals present in the Aquous extracts of leaf of *carica papaya*, *Azadirachta indica*, *Ocimum sanctum*, *Cymbopogon citratus*, *Mentha spicata*, *Aloe vera*, *Vitex negundo*, *Plectranthus amboinicus*, *Senna auriculata*, *Manilkara zapota*. *Ocimum sanctum*, *Azadirachta indica*, bulb of *Allium sativum* and flower of, *Targetes erecta* contain most of the phyto chemical components andin Ethyl alcohol extract mostly *Ocimum sanctum* gave maximum result. The phytochemicals of the plants serve as huge storage of compounds that have biological action. Alkaloids, saponins, and tannins are known to possess medicinal and larvicidal properties.

In our phytochemical analysis of 12 plants, *Azadirachta indica* in Aqueous extract show the highest result. Which have Alkaloid, glycoside, saponin, phytosterols, flavonoid, protein and diterpenes and *Allium sativum* in ethyl alcohol extract show the lowest result which contain glycoside, saponin, phytosterol, flavonoid and diterpenes. Phytochemical screening and antimicrobial activity of leaf extract of *Wrightia tomentosa* revealed that the plant contains alkaloids, steroids, tannins, etc. and showed antimicrobial activity.

**Table 1** Phytochemical analysis of selected medicinal plants

Test	<i>Ocimum sanctum</i>		<i>Mentha Spicata</i>		<i>Azadirachta indica</i>		<i>Cymbopogon citratus</i>		<i>Senna auriculata</i>		<i>Targetes Erecta</i>		<i>Vitex negundo</i>		<i>Plectranthus Auriculata</i>		<i>Manilkara. zapota</i>		<i>Aloe vera</i>		<i>Carica papaya</i>		<i>Allium sativum</i>	
	W	E	W	E	W	E	W	E	W	E	W	E	W	E	W	E	W	E	W	E	W	E	W	E
AL	+	-	+	+	+	-	+	-	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-
CA	+	-	-	-	+	+	-	+	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-
GL	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GL	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-
SA	-	+	+	+	+	+	+	-	+	-	+	-	+	-	-	+	-	-	-	-	+	-	-	-
SA	-	+	+	+	+	+	+	-	+	-	+	-	+	-	-	-	-	-	-	-	+	-	+	-
PH	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	-	-
PH	-	-	+	-	+	+	-	+	+	-	-	-	+	-	+	-	-	+	-	-	+	-	-	+
FL	+	+	+	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	-	-	-	+	-
FL	+	-	+	-	+	-	+	-	+	+	-	-	-	+	+	-	+	-	-	+	-	-	-	+
PR	+	+	+	-	+	-	+	-	+	-	-	-	+	-	+	+	+	-	+	-	+	-	-	-
PR	+	-	+	-	+	-	-	-	+	-	+	-	+	+	-	-	+	-	+	-	+	-	-	+
DI	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	-	+	-	+	-	-

AL	ALKALOIDS
CA	CARBOHYDRATES
GL	GLYCOSIDES
SA	SAPONINS
PH	PHYTOSTEROLS
FL	FLAVONOIDS
PR	PROTEIN
DI	DITERPENES

In addition, compounds such as flavonoids, alkaloids, tannins and saponins in the plants are responsible for the insecticidal and toxicity to other animals. Saponins are known by their toxicity to harmful insects. Saponins isolated from *Achyranthes aspera* through bioassay guided fractionation possessed a larvicidal efficacy against *A. aegypti* and *C. Quinquefasciatus*. Moreover, Flavonoids isolated from water extracts of *Annona squamosa* is effective as insecticides against mosquito killing 80% of *C. Chinensis* (Srinivas *et al.* 2013).

Phytochemical screening revealed the presence of alkaloids, flavonoids in the leaf and bark extracts of *Jatropha curcas* while the leaf and bark/stem extracts of *Citrus grandis* and *Tinospora rumphii* are rich in alkaloids, saponins, tannins, flavonoids and steroids. These compounds are known to possess insecticidal and larvicidal properties causing the mortality of insects and other pests. All plant extracts showed significant larvicidal activity against *A. aegypti* mosquito larvae at 0.05 level of significance. *Tinospora rumphii* leaf extract is the most effective mosquito larvicide which is manifested by the highest percentage mortality on the larvae of 90% and 93% after 24 and 48 hours respectively; with an LC<sub>50</sub> and LC<sub>90</sub> values of 10 mg/mL and 46 mg/mL respectively after 48 hours of exposure. *Citrus grandis* bark and *Tinospora rumphii* stem extracts showed a significant difference on the increased of the mortality of mosquito larvae with increasing concentrations of the plant extracts at 0.05 level of significance. The high activities of the three plants differ according to the plant species and part used which is supported by the presence of several bioactive chemicals (Maurya *et al.*, 2007 and Kassir *et al.*, 1989)

The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. For instance, Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Nobori *et al.*, 1994). Several workers have reported the analgesic (Harborne, 1998) antispasmodic and antibacterial properties of alkaloids. Glycosides are known to lower the blood pressure according to many reports.

The terpenoids have been shown to decrease blood sugar level in animal studies. Saponins has the property of precipitating and coagulating red blood cells. Flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-llergic, anti-inflammatory, anti-microbial and anti-cancer activities (Aiyelaagbe and Osamudiamen, 2009).

Tannins and saponins are present in leaves extracts but absent in leaves extracts but absent in stem bark and flower extracts. Flavonoids are present in stem bark and flower but absent in leaves. The presence of flavonoids indicates the natural occurring phenolic compound, with beneficial effects in the uman diet as antioxidants and neutralizing free radicals. Tannins are group of polymeric phenolic compound and cause local tumors. Teroenoids and steroids were detected in *Moringa pterygosperma* which were reported to be active against antibacterial activity Saponins have the properties of precipitating and coagulating red blood cells, anti-inflammatory. Alkaloids are used in medicines for reducing headache he and fever. This are attributed for anti-bacterial and analgesic properties (Sheeran 2006 and Medhi 2010). Alkaloid has important biological property like cytotoxicity and re used in allophatic systems. Steroids are present in ethanol clove, ethanol pepper, acetone pepper and saffron extracts. Steroids and Sterols are great importance in pharmacy as they possess compounds like sex hormones and can be used for drug production (Okwu, 2001). The larvicidal effects of leaf extracts of *carica papaya*, *Azadirachta indica*, *Ocimum sanctum*, *Cymbopogon citratus*, *Mentha spicata*, *Aloe vera*, *Vitex negundo*, *Plectranthus amboinicus*, *Senna auriculata*, *Manilkara zapota*, Flower extract of, *Targetes erecta*, and bulb extract of *Allium sativum* were tested on the larvae of the dengue-vectors *Aedes aegypt* and *Anopheles*. Phytochemical screening of the extracts was conducted to determine the active toxic compounds. Various concentrations (10 ppm, 20 ppm and 50 ppm) of the plant extracts were tested against third instar larvae of *A. aegypti* and *Anopheles*.

Table-2 and 3 shows that the testing the Aqueous and Ethyl alcohol extract of leaves of *Carica papaya*, *Azadirachta indica*, *Ocimum sanctum*, *Cymbopogon citratus*, *Mentha spicata*, *Aloe vera*, *Vitex negundo*, *Plectranthus amboinicus*, *Targetes erecta*, *Manilkara zapota*, flower of *Senna auriculata* and bulb of *Allium sativum* in the various amount 10 ppm, 20 ppm and 50 ppm separately in both the *Aedes* and *Anopheles* larvae in the container and take the result for 24 and 48 hours. Show the result that in 48 hours of 20 ppm aqueous extract of *Ocimum sanctum*, and 50 ppm of *Ocimum sanctum*, *Mentha spicata*, *Azadirachta indica*, *Cymbopogon citratus*, *Senna auriculata*, *Targetes erecta*, and *Aloe vera* in *Aedes* show the 100% mortality and in Ethyl alcohol of 20 ppm extract of *Ocimum sanctum*, *Mentha spicata* and 50 ppm extract of *Carica papaya*, *Azadirachta indica*, *Ocimum sanctum*, *Mentha spicata*, *Aloe vera*, *Vitex negundo*, *Plectranthus amboinicus*, *Targetes erecta*, *Manilkara zapota*, and *Allium sativum* in *Aedes* show 100% mortality and in 48 hours of aqueous extract of *Azadirachta indica*, *Ocimum sanctum*, *Cymbopogon citratus*, *Mentha spicata*, *Senna auriculata*, *Targetes erecta* and *Aloe vera* in *Anopheles* show 100% mortality and in 50 ppm of Ethyl alcohol extract of *Carica papaya*, *Azadirachta indica*, *Ocimum sanctum*, *Cymbopogon citratus*, *Mentha spicata*, *Vitex negundo*, *Plectranthus amboinicus*, *Targetes erecta*, *Manilkara zapota*, *Senna auriculata* and *Allium sativum* in *Anopheles* show 100% mortality. Conclusion, an attempt has been made to evaluate the role of plant extracts in mosquitoes larvicidal activity. The results reported in this study open the possibility of further investigation of the efficiency of the larvicidal properties of natural product extracts.

activities which could play an important role in the search for new biocompounds. Secondary metabolites produced in plants for its protection against microorganisms and predator insects are natural candidates for the discovery of new products to combat *A.aegypti* and *Anopheles*. Several studies have focused on natural products for controlling *Aedes* mosquitoes as insecticides and larvicides, but with varied results (Anitha Rajasekaran and Geethapriya Duraikannan., 2012).

In the present study the 20 ppm aqueous extract of *Ocimum sanctum*, and 50 ppm of *Ocimum sanctum*, *Mentha spicata*, *Azadirachta indica*, *Cymbopogon citratus*, *Senna auriculata*, *Targetes erecta*, and *Aloe vera* in *aedes* show the 100% mortality and in Ethyl alcohol of 20 ppm extract of *Ocimum sanctum*, *Mentha spicata* and 50 ppm extract of *Carica papaya*, *Azadirachta indica*, *Ocimum sanctum*, *Mentha spicata*, *Aloe vera*, *Vitex negundo*, *Plectranthus amboinicus*, *Targetes erecta*, *Manilkara zapota*, and *Allium sativum* in *Aedes* show 100% mortality and in 48 hours of aqueous extract of *Azadirachta indica*, *Ocimum sanctum*, *Cymbopogon citratus*, *Mentha spicata*, *Senna auriculata*, *Targetes erecta* and *Aloe vera* in *Anopheles* show 100% mortality and in 50 ppm of Ethyl alcohol extract of *Carica papaya*, *Azadirachta indica*, *Ocimum sanctum*, *Cymbopogon citratus*, *Mentha spicata*, *Vitex negundo*, *Plectranthus amboinicus*, *Targetes erecta*, *Manilkara zapota*, *Senna auriculata* and *Allium sativum* in *Anopheles* show 100% mortality after 48hrs of incubation. Moreover behavioral changes were observed in the movement of the larvae. These effects may be due the presence of neurotox in compounds in plant extracts. No behavioral changes were obtained in control group.

**Table 2** Antilarvicidal activity of selected medicinal against aqueous extract

Plant name	<i>Aedes</i> (Percentage)						<i>Anopheles</i> (Percentage)					
	24 hours			48 hours			24 hours			48 hours		
	10 ppm	20 ppm	50 ppm	10 ppm	20 ppm	50 ppm	10 ppm	20 ppm	50 ppm	10 ppm	20 ppm	50 ppm
<i>O.sanctum</i>	20	90	90	60	100	100	0	0	20	100	100	100
<i>M.spicata</i>	40	90	90	60	70	100	40	60	60	70	100	100
<i>A.indica</i>	0	0	40	70	90	50	70	80	90	90	100	100
<i>C.citratus</i>	80	90	90	90	100	100	80	90	100	80	100	100
<i>S.auriculata</i>	90	100	100	70	100	100	80	80	90	90	90	100
<i>T. erecta</i>	40	60	60	40	70	90	60	60	40	80	90	90
<i>V.negundo</i>	0	40	90	70	90	100	80	80	90	80	90	90
<i>P.amboinicus</i>	80	80	90	90	80	100	80	90	100	90	100	100
<i>M. zapota</i>	0	0	20	40	50	60	40	40	50	80	70	80
<i>A.vera</i>	40	60	60	60	60	90	90	20	80	80	90	100
<i>C.papaya</i>	0	40	60	60	50	100	40	60	60	60	70	80
<i>A.sativum</i>	40	40	50	60	60	70	0	20	80	70	80	90

**Table 3** Antilarvicidal activity of selected medicinal against Ethyl alcohol extract

Plant name	<i>Aedes</i> (Percentage)						<i>Anopheles</i> (Percentage)					
	24 hours			48 hours			24 hours			48 hours		
	10 ppm	20 ppm	50 ppm	10 ppm	20 ppm	50 ppm	10 ppm	20 ppm	50 ppm	10 ppm	20 ppm	50 ppm
<i>O.sanctum</i>	40	70	90	70	90	100	20	60	70	50	100	100
<i>M.spicata</i>	20	50	80	40	70	100	10	40	70	60	90	100
<i>A.indica</i>	10	30	50	40	60	100	30	50	70	50	80	100
<i>C.citratus</i>	10	30	40	40	70	90	30	50	90	70	90	100
<i>S.auriculata</i>	20	30	50	70	90	100	10	60	80	30	50	100
<i>T. erecta</i>	20	40	60	40	50	80	10	50	80	60	80	100
<i>V.negundo</i>	40	70	100	60	100	100	20	50	80	70	90	100
<i>P.amboinicus</i>	10	30	50	50	70	100	0	40	90	40	70	100
<i>M. zapota</i>	40	60	80	70	90	100	20	50	70	40	70	100
<i>A.vera</i>	40	70	90	60	90	100	20	60	70	50	90	90
<i>C.papaya</i>	40	50	80	50	80	100	20	60	80	60	90	100
<i>A.sativum</i>	10	50	70	40	80	100	30	50	90	80	70	100

The result of this screening show that plant extracts represents a rich source of bioactive molecule with often specific

Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent and ovipositor attractant and

have different activities observed by many researchers. However, insecticides of plant origin have been extensively used on agricultural pests and to a very limited extent, against insect vector of public health importance.

Crude extracts or isolated bioactive phytochemicals from the plant could be used in stagnant water bodies which are known to be the breeding grounds for mosquitoes. However, further studies on the identification of the active principals involved and their mode of action and field trials are usually needed to recommend any of these plant materials as an anti-mosquito product used to combat and protect from mosquitoes in a control program. Plant could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive chemicals and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. Further analysis is required to isolate the active principles and its mode of action in inhibiting the developmental stages in *Aedes aegypti*. The phytochemicals of medicinal extracts can be well utilized for preparing biocides or insecticidal formulation. In view of growing concern of mosquito nuisance and vector borne diseases in urban and tribal populations, the use of plant extracts should be promoted to reduce the toxic load of insecticides on the environment. Synthetic chemicals, which were harmful in the long term, did not provide absolute protection. Application of chemical insecticides to control vector mosquitoes resulting, in problems like residual effects, vector resistance and toxicity to the components of the ecosystem alternatively, plant extracts could be effectively employed in mosquito control programs (Anitha and Geethapriya, 2012)

Plant sources possess a wide range of pharmaceutical and insecticidal properties. The insecticides of plant origin did not disturb the environment. Besides reducing the cost-factor plants exhibit different, degrees of toxicity to the different stages and conditions of mosquitoes such as larvicidal, pupicidal, adulticidal, growth and reproduction inhibition, repellent and ovipositional deterrents. The very high activity of the extracts especially *Albizia amara*, *Areca catechu*, *Leucas aspera* and *Ocimum sanctum* against the larvae of *Anopheles stephensi* suggested that the methanol extracts might be used directly as larvicidal agents in small volume aquatic habitats or breeding sites of around human dwellings (Srinivas *et. al.* 2013).

The 100% mortality might be due to the chemical constituents present in the leaf extracts that arrest the metabolic activities of the larvae, which caused higher percentage of mortality. The increase in turbidity at higher concentration might block the oxygen depletion to the larvae. The active fractions of *Leucas aspera* were found to be significant toxic to fourth instar larvae. The active fractions of *Ocimum sanctum* were also found to be toxic. Out of the ten plants tested *Adathoda vasica* and *Musa paradisiaca* was found to be less toxic when compared to the other plants. The varying results were probably due to the differences in levels of toxicity among the insecticidal ingredients of each plant. Treatment with leaf extracts of *Leucas aspera* and *Ocimum sanctum* on larvae exhibited high mortality, especially during the moulting process. Such processes were under the influence of the ventral nerve cord neurosecretory cells, which release the tanning hormone. These extracts might have an inhibiting influence on

such cells or might act directly on epidermal cells which were responsible for the production of enzymes for the tanning or cuticular oxidation process. This was further evidenced by the fact that many of the larvae treated with higher concentration did not successfully moult to pupae. In the present study, the treatment with medicinal plants rendered the *Anopheles stephensi* larvae inactive and motionless. Higher degree of disturbance in normal behavior of the larvae was observed in treatments of *Adathoda*, *Albizia*, Banana, *Eucalyptus*, *Phyllanthus*, *Leucas* and Pepper suggesting that these botanicals affect the normal behavioural physiology and biology in a significant manner (Anitha Rajasekaran and Geethapriya Duraikannan., 2012, Mansour *et.al.*, 2000; Trabulsi *et.al.*, 2012).

In the present study a significant reduction of carbohydrates was observed when the larvae were treated with high dose. The carbohydrates were found to be reduced in the treated larvae. The present results had shown that the treatment caused a decrease in the DNA and RNA content when compared to the control. In the present investigations the plant extracts significantly increased the larval mortality and caused less food consumption. These results indicated that a certain finite amount of the plant extracts would be sufficient for the enhancing effect. Chemical analysis of the insects indicated that the carbohydrate was significantly affected due to the treatments resulting in overall collapse of the metabolism and growth. The present findings have important implications in the practical control of mosquito larvae in the polluted aquatic ecosystem. The plants studied are available in large quantities. These extracts are easy to handle, inexpensive and safe natural products for mosquito control. The extracts of tulsi can also be used for disinfecting water. In view of residue problems in the environment and the development of insect resistance to synthetic insecticides like DDT and other chlorinated hydrocarbons, the recent trend is to explore plants to obtain extracts that are safe for non target animals and do not pose any residue problem but are still able to suppress pest populations. Though several compounds of plant origin have been reported as insecticides, larvicides, there is a wide scope for the discovery of more effective plant products. Further research undoubtedly will lead to improved formulations with enhanced activity which may eventually become environmentally acceptable and replace objectionable conventional insecticides for mosquito control (Bower *et.al.*, 1995; Rajkumar and Jebanesen, 2009; Sheeran *et.al.*, 2006 and Kassir *et.al.*, 1989).

## CONCLUSION

The overall view that, The phytochemicals present in the Aqueous extracts of flower, bulb and leaf of *carica papaya*, *Azadirachta indica*, *Ocimum sanctum*, *Allium sativum*, *Cymbopogan citratus*, *Mentha spicata*, *Aloe vera*, *Vitex negundo*, *Senna auriculata*, *Plectranthus amboinicus*, *Targetes erecta* and *Manilkara zapota*. *Ocimum sanctum*, *Azadirachta indica* and *Senna auriculata* leaves contain most of the phytochemical components. However, in Ethyl alcohol extract mostly *Ocimum sanctum* gave maximum result.

Testing the Aqueous and Ethyl alcohol extract of leaves of *Carica papaya*, *Azadirachta indica*, *Ocimum sanctum*, *Cymbopogan citratus*, *Mentha spicata*, *Aloe vera*, *Vitex negundo*, *Plectranthus amboinicus*, *Targetes erecta*, *Manilkara*

zapota, flower of *Senna auriculata* and bulb of *Allium sativum* in the various amount 10 ppm, 20 ppm and 50 ppm separately in both the *Aedes* and *Anopheles larvae* in the container and take the result for 24 and 48 hours. Show the result that in 48 hours of 20 ppm aqueous extract of *Ocimum sanctum*, and 50 ppm of *Ocimum sanctum*, *Mentha spicata*, *Azadirachta indica*, *Cymbopogon citratus*, *Senna auriculata*, *Targetes erecta*, and *Aloe vera* in *aedes* show the 100% mortality. In Ethyl alcohol of 20 ppm extract of *Ocimum sanctum*, *Mentha spicata* and 50 ppm extract of *Carica papaya*, *Azadirachta indica*, *Ocimum sanctum*, *Mentha spicata*, *Aloe vera*, *Vitex negundo*, *Plectranthus amboinicus*, *Targetes erecta*, *Manilkara zapota*, and *Allium sativum* in *Aedes* show 100% mortality and in 48 hours of aqueous extract of *Azadirachta indica*, *Ocimum sanctum*, *Cymbopogon citratus*, *Mentha spicata*, *Senna auriculata*, *Targetes erecta* and *Aloe vera* in *Anopheles* show 100% mortality and in 50 ppm acetone extract of *Carica papaya*, *Azadirachta indica*, *Ocimum sanctum*, *Cymbopogon citratus*, *Mentha spicata*, *Aloe vera*, *Vitex negundo*, *Plectranthus amboinicus*, *Manilkara zapota*, *Senna auriculata* and *Allium sativum* show 100% mortality and in 50 ppm of Ethyl alcohol extract of *Carica papaya*, *Azadirachta indica*, *Ocimum sanctum*, *Cymbopogon citratus*, *Mentha spicata*, *Vitex negundo*, *Plectranthus amboinicus*, *Targetes erecta*, *Manilkara zapota*, *Senna auriculata* and *Allium sativum* in *Anopheles* show 100% mortality. The results reported here open the possibility of further investigations of efficacy on their larvicidal of natural products extracts. Further research undoubtedly will lead to improved formulations with enhanced activity which may eventually become environmentally acceptable and replace objectionable conventional insecticides for Mosquito control.

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**How to cite this article:**

Jeyapreethi Selvam and Muthuselvam Durai.2018, Phytochemical Analysis And Larvicidal Activity of Aqueous And Ethanol Extract of Selective Medicinal Plants Against Malaria And Dengue Vector. *Int J Recent Sci Res.* 9(5), pp. 27113-27119.  
DOI: <http://dx.doi.org/10.24327/ijrsr.2018.0905.2190>

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