Research Article (Open access)

Comparative Studies on *in- vitro* Phytochemicals Analysis and Larvicidal Efficacy of Medicinal Plant Extracts against *Culex quinquefasciatus*

S. Narendiran[1]*, Janani. D[2], Keerthana. M[2], Nivethitha K. S[2], Nirmala Devi. S[2], Padmavathy. S[2], Supraja. T. S[2], Sayeedur Rahman. H[2], Velvizhi. R[2], Swathi. N[3], Yasaswini. K. G[3]

[1]Department of Biotechnology, Sree Sastha Institute of Engineering and Technology, Chembarambakkam, Chennai, India
[2]Department of Biotechnology, VIT University, Vellore, India
[3]Department of Biotechnology, Sathyabama University, Chennai, India

[*]**Address for Correspondence:** S. Narendhiran, Research analyst, Food and Technology, Innovative Health Care India Pvt Ltd, Chennai, India

Received: 17 September 2016/Revised: 04 October 2016/Accepted: 22 October 2016

ABSTRACT- Mosquitoes transmit human diseases, causing millions and millions of deaths every year Mosquito borne diseases are one of the most serious public health problems in the developing countries. It can be controlled by using repellent, causing larval mortality and the development of resistance to chemical insecticides resulting in rebounding vectorial capacity. Plants may be alternative sources of mosquito control agents. Medicinal plants extracts of *Vitex negundo*, *Azadirachta indica* and *Eucalyptus tereticornis* were tested for their larvicidal activity against *Culex quinquefasciatus*. There are four different solvents were used (Petroleum-ether, Ethanol, Acetone and Hexane extract) for the preparation of crude extracts from the plant leaves. The larval mortality of second and third instar larvae *C. quinquefasciatus* after 24 hour to 48 hour of treatment were observed separately in control,100, 200,300,400 and 500 ppm concentrations of the leaf extract. The seven different solvent extract of *Vitex negundo* showed good larvicidal activity.

Key-words- Larvicidal, Medicinal plant extracts, Phytochemicals Analysis, *Culex quinquefasciatus*

----------IJLSSR----------

INTRODUCTION

The mosquito is the principal vector of many of the vector borne diseases affecting human beings and other animals. Several mosquito species belonging to genera Anopheles, Culex and Aedes are vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue fever, dengue hemorrhagic fever and yellow fever. Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concern, which initiated a search for alternative control measures. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents One of the approaches for control of these mosquito borne diseases is the interruption of disease transmission by either killing, preventing mosquitoes to bite human beings (by using repellents) or by causing larval mortality in a large scale at the breeding centers of the vectors. This study is concerned with the using of such effective plant source against the larval of Mosquito. Blood-feeding female mosquitoes are responsible for the intolerable biting nuisance and transmission of a large number of diseases such as malaria, yellow fever, dengue, filariasis, chiken-gunya, and encephalitis. They cause serious health problems to humans and present obstacles to the socioeconomic development of developing countries, particularly in the tropical region [1]. In addition to mortality, vector borne diseases cause morbidity of millions of persons resulting in loss of man-days and causing economic loss [2].

There are 300 species of mosquitoes belonging to 41 genera; all contained in the family Culicidae.

Access this article online	
Quick Response Code:	Website: www.ijlssr.com
	crossref DOI: 10.21276/ijlssr.2016.2.6.15

Aedes aegypti, a vector of dengue is now endemic and found to be widely distributed in the tropic and subtropics. Synthetic chemical larvicides are parts of the world. But many of these chemicals are toxic to human, plant and animal life and insecticides resistance can be problematic in maintaining control, especially with organophosphate and pyrethroid larvicides [3]. Therefore, a more efficient approach to reduce the population of mosquitoes would be to target the larvae. Mosquitoes are ecologically important components of the aquatic and terrestrial food chain, then they are the most important group of insects in terms of public health importance, and thus, appropriate control programs are justified. Until a few years ago, only the adults were sprayed, but now, it is well known that a more efficient way to reduce mosquito populations is to target the larvae [3-4].

MATERIALS AND METHODS

The list of plant leaves included in this study was

1. *Vitex negundo* (Nochi)
2. *Azadirachta indica* (neem)
3. *Eucalyptus tereticornis* (Thailam)

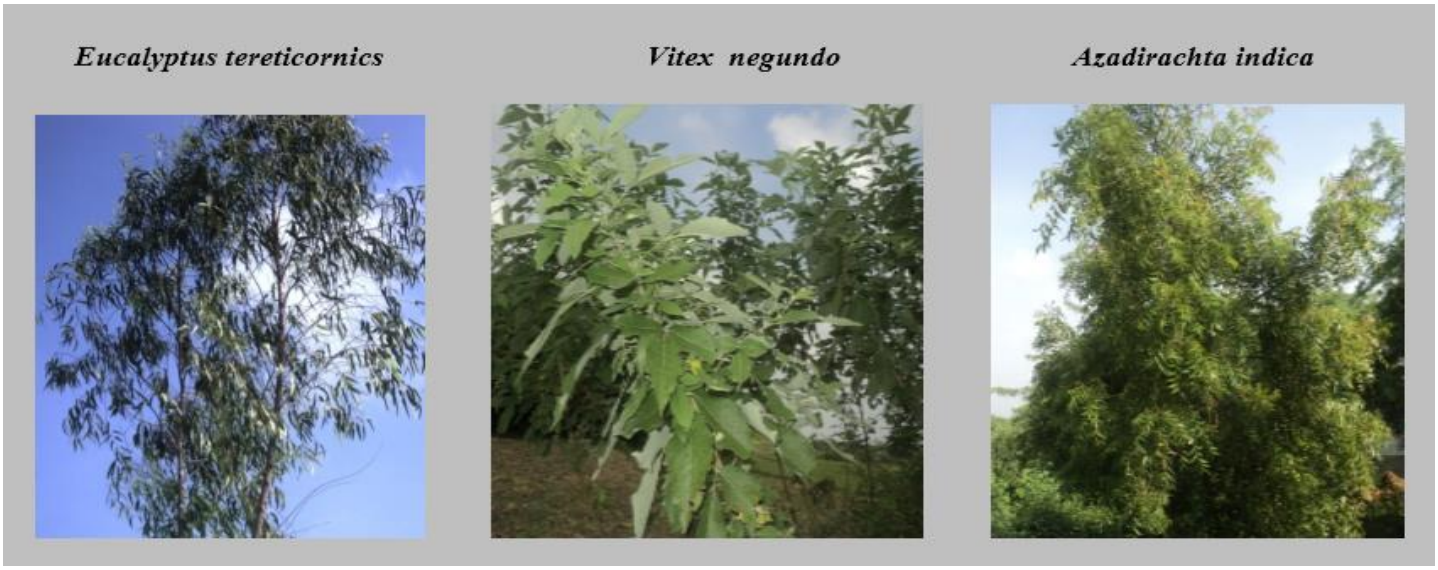

Fig. 1: Medicinal plants

COLLECTION OF MOSQUITO LARVAES

The mosquito larvae (*Culex quinquefasciatus*) were collected from the Chembarambakkam Lake, situated near Chennai-Bangalore highway Tiruvallur district of Tamil Nadu, India. The larvae were collected in a container and transferred to the laboratory immediately. From these larvae, unwanted large size larvae and pupae were collected and discarded from the remaining medium sized larvae second and third instar larvae alone were collected for the larvicidal bioassay. Feed is supplied to the mosquito larvae for its growth.

Preparation of Medicinal Plant Extracts

10 grams of each plant leaves powder was taken separately and 100ml of solvents (Ethanol, Petroleum ether, Hexane and Acetone extract)was added and kept overnight in the shaker. The extract is filtered again the solvent is added and kept at room temperature in the shaker for 7 hours. In this way the plant extract is collected and stored in the air tight container for future use.

Test for Carbohydrates

1ml of different crude extract was dissolved in 10ml of distilled water and filtered. The filtrate underwent Molish's test to confirm the presence of carbohydrates [5].

Molish's Test

The filtrate was treated with 2ml of concentrated sulphuric acid along the sides of the test tube. Appearance of violet colour ring at the junction of the two liquid shows the presence of carbohydrates.

Test of Glycosides

A small portion of different crude extract was hydrolyzed with hydrochloric acid for few hours on water bath and the hydrolysate was collected [5].

Fehling's Test

2ml of extract was taken in a test tube. 1ml of Fehling's A solution and 1ml of Fehling's B solution was added to the extract, mixed well and boiled. Appearance of yellow or red colour precipitate indicates the presence of glycosides (reducing sugar).

Test for Proteins
A small portion of crude was dissolved in few ml of distilled water and it was subjected to Xantho protein test to confirm the presence of protein [5].

Xantho Test
Take 3ml of extract to which 1ml of concentrated nitric acid was added. A white precipitate was obtained. The solution was heated for 1 minute and cooled under the tap water. 40% of NaOH was added to the solution. Appearance of orange colour indicates the presence of protein.

Test for Phenolic compounds and Tannins
A small portion of crude extract was dissolved in few ml of distilled water and subjected to $FeCl_3$ test [5].

$FeCl_3$ Test
Few ml of extract 5% $FeCl_3$ was added. Appearance of violet colour indicates the presence of phenolic compounds and tannins.

Test for Flavonoids
The crude extract was treated with concentrated sulphuric acid. Appearance of yellowish orange colour indicates the presence of anthocyanin, on further adding yellow turns to orange which indicates the presence of flavones, on further adding turns to crimson which indicates the presence of flavonones [5].

Test for Terpenoids
2ml of crude extract was dissolved in 2ml of chloroform to which 2ml of sulphuric acid was added and the heated for 2 minutes. Appearance of grayish colour indicates the presence of terpenoids.

Test for Phlobatannins
2ml of extract was taken to which 2ml of 1% HCl was added and boiled. Formation of red precipitate indicates the presence of phlobatannins [5].

LARVICIDAL BIOASSAY
Initially 12.5 mg crude extract of petroleum ether solvent of each solvent extract was taken and dissolved in 1ml of acetone in an eppendorf. Then the dissolved crude extract was mixed in container containing 50 larvae in 100ml of water. Every 24 hours the mortality rate was noted and reading was taken. The dead larvae were taken out at every 24 hours since it may leads to contamination of the water. The readings were taken for 2 days (48hours) then 0.25% (250ppm) concentration from each plant crude extract was introduced into containers containing larvae. Similarly for this reading wastaken for every 24 hours for 2 days. Then 0.50% concentration (500ppm) of crude extract was introduced and reading was noted for every 24hrs for two days [6].

Qualitative Analysis of Phytochemicals
The phyto constituents detected in the plant extract could be responsible for their antioxidant and antimicrobial activity.

Table 1: Qualitative analysis of phytochemicals in various extracts of *Vitex negundo*

Phytochemicals/ Extracts	Ethanol extract	Petroleum ether extract	Hexane extract	Acetone Extract
Alkaloids	-	+	-	+
Steroids	+	+	+	-
Triterpenoids	+	-	-	+
Flavanoids	+	+	+	+
Tannins	-	-	-	+
Phenols	+	+	-	-
Glycosides	+	-	+	+
Saponins	-	-	-	-
Phlobotannins	+	+	+	-
Anthraquinones	+	+	-	+

(+): Presence of phytochemicals
(-): Absence of phytochemicals

Table 2: Qualitative analysis of phytochemicals in various extracts of *Azadirachta indica*

Phytochemicals/ Extracts	Ethanol extract	Petroleum ether extract	Hexane extract	Acetone Extract
Alkaloids	-	+	-	+
Steroids	+	+	+	-
Triterpenoids	+	-	-	+
Flavanoids	+	+	-	+
Tannins	+	-	+	-
Phenols	-	+	-	-
Glycosides	+	-	+	+
Saponins	-	-	+	-
Phlobotannins	+	+	+	-
Anthraquinones	+	+	-	+

(+): Presence of phytochemicals
(-): Absence of phytochemicals

Table 3: Qualitative analysis of phytochemicals in various extracts of *Eucalyptus tereticornis*

Phytochemicals/Extracts	Ethanol extract	Petroleum ether extract	Hexane extract	Acetone Extract
Alkaloids	-	+	-	+
Steroids	+	+	+	-
Triterpenoids	+	+	-	-
Flavanoids	+	+	+	+
Tannins	-	-	-	+
Phenols	+	+	-	+
Glycosides	+	+	+	+
Saponins	-	+	-	+
Phlobotannins	+	+	+	-
Anthraquinones	+	-	-	+

(+): Presence of phytochemicals
(-): Absence of phytochemicals

RESULT AND DISCUSSION

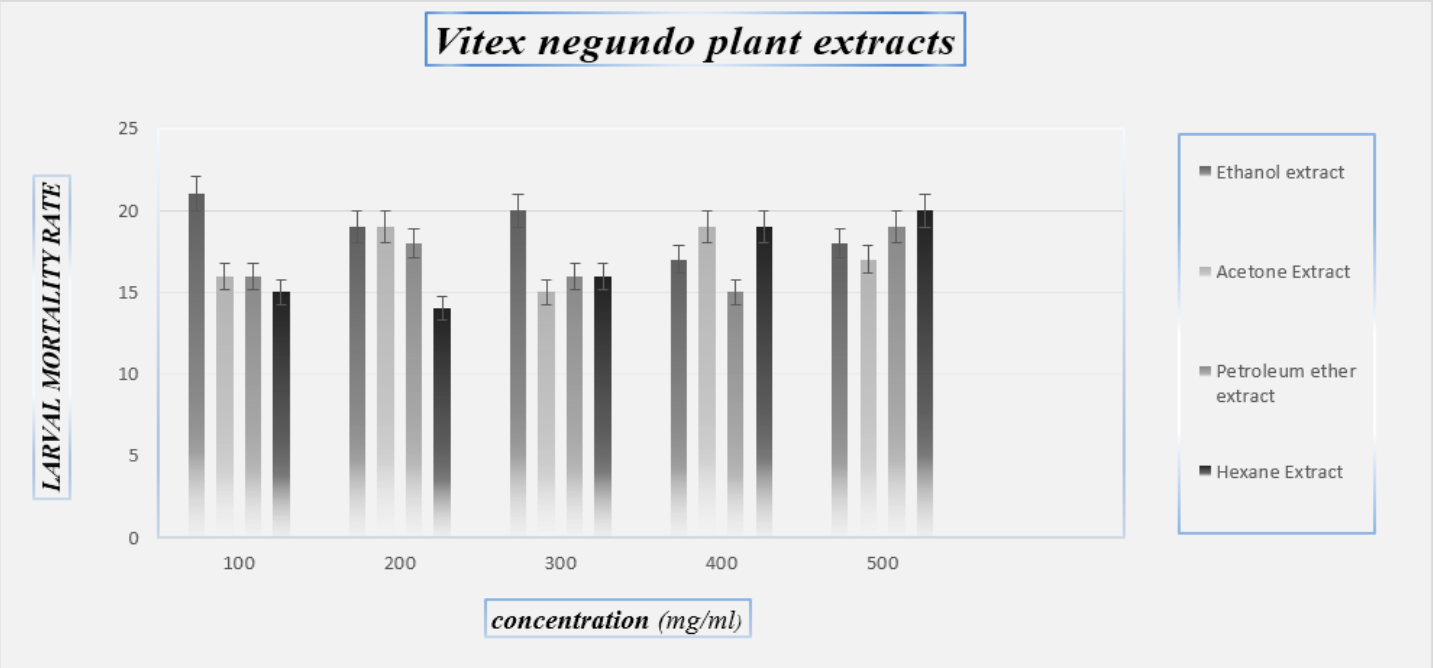

Fig. 2: Larvae mortality rate on Vitex negundo plant

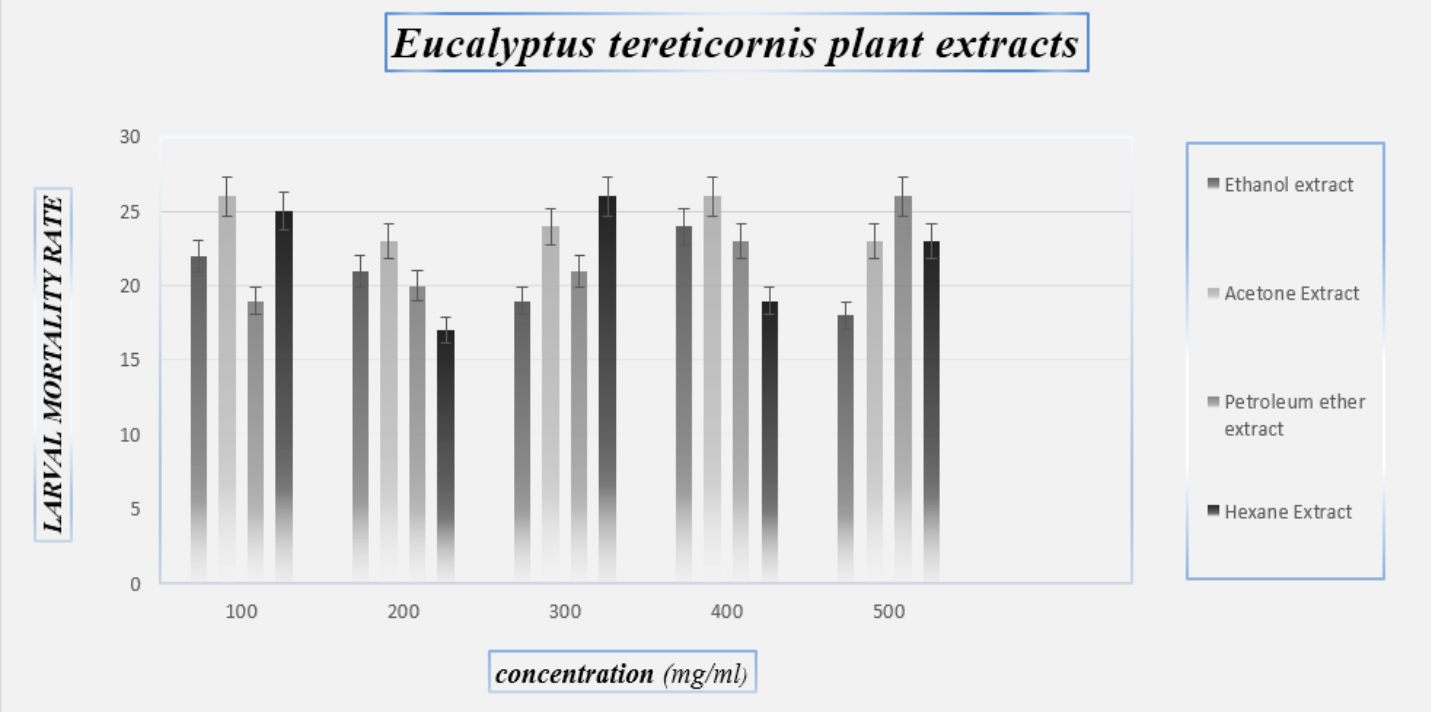

Fig. 3: Larvae mortality rate on *Eucalyptus tereticornis* plant

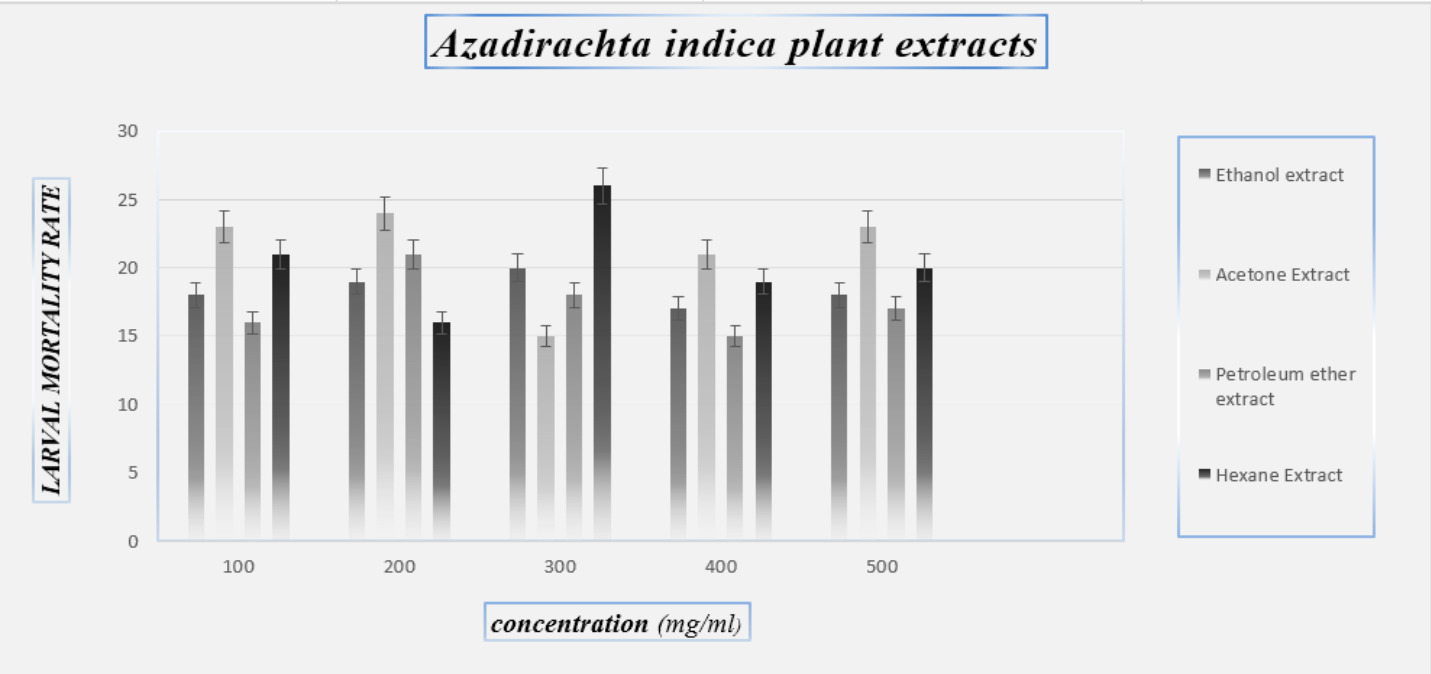

Fig. 4: Larvae mortality rate on *Azadirachta indica* plant

Larvicidal activities of seven medicinal plant extracts were studied with the control, 100, 200, 300, 400 and 500 ppm concentrations. The present study assessed the role of larvicidal activities of Medicinal plants extracts of *Vitex negundo* and *Azadirachta indica* and *Eucalyptus tereticornis* by Solvents Petroleum ether, Hexane, Acetone and Ethanol against the larvae of malaria vector *Culex quinquefasciatus.* The activity of crude extracts of the medicinal plant is noted. The seven different solvents were tested against *Culex quinquefasciatus* and larval mortality percent was observed .through statistical analysis it was analyzed that all plant extracts showed moderate toxic effect on larval. However, the highest mortality rate was found by the *Eucalyptus tereticornis* plant extracts.

CONCLUSIONS

An insecticide does will not cause high mortality on target larvae but may serve medicinal plants extracts as suitable alternatives to synthetic insecticides plant extract are more safer, inexpensive, reduce dependence on imported products (synthetic) and are readily available in many region of the world. The findings of the present

investigation revealed that *Eucalyptus tereticornis* has good larvicidal activity against *Culex quinquefasciatus* and *Azadirachta indica* showed poor mortality against the *Culex quinquefasciatus* larvae. These crude extracts can be effectively used in the control of mosquitoes by replacing the chemical pesticides which cause environmental pollutions and other burdens. Statistically *Vitex negundo* had high larvicidal efficacy. These Medicinal plants extracts are the best alternate product against the control of mosquito's vector. It can be replace the chemical pesticides which cause environmental pollutions and other health problems.

REFERENCES

[1] Senthilkumar A, Kannathasan K, Venkatesalu V. Chemical constituents and larvicidal property of the essential oil of *Blumeamollis* (D. Don) Merr against Culex quinquefasciatus. Parasitol Res., 2008; 103: 959-62.

[2] Dhiman RC, Pahw S, Dhillon GP, Dash AP. Climate change and threat of vector-borne diseases in India: are we prepared? Parasitol. Res., 2010; 106(4): 763-73.

[3] Rahuman AA, Bagavan A, Kamaraj C, Saravanan E, Zahir AA, Elango G. Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasito.l Res.* 2009; 104(6): 1365-72.

[4] Govindarajan M, Jebanesan A, Pushpanathan T. Larvicidal and ovicidal activity of Cassia fistula Linn. Leaf extract against filarial and malarial vector mosquitoes. *Parasitol Res 2008*; 102(2): 289-92.

[5] Arun J, Pennarasi, Prasanna. M, SundaraMahalingam, Narendhiran, Alagesan, Esaipriyan. Screening of Phytochemical Constituents towards Antimicrobial Potency of Hygrophilaschulli. A J. Biotechnol. 2231-3826, Special Issue, 2013

[6] Maheswaran R, Sathish S, Ignacimuthu. S, Larvicidal activity of Leucasaspera against the larvae of *Culex quinquefasciatus* and *Aedes aegypti*. Int. J. Integrative. Biol., 2008; 2(3): 214-17.

[7] Su T, Mulla MS. Ovicidal activity of neem products (*azadirachtin*) against *Culex tarsalis* and *Culex* quinquefasciatus (Diptera: *Culicidae*). J. Am. Mosq. Control. Assoc., 1998; 14: 204-49.

[8] Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: a review. J. Am. Mosq. Control. Assoc., 1991;7 : 210-37.

[9] Dhar R, Dawar H, Garg S,Basir SF, Talwar GP. Effect of volatiles from Neem and other natural products on gonotrophic cycle and oviposition of Anopheles stephensi and An. culicifacies (Diptera: Culicidae). J. Med. Entomol., 1996; 33: 195-201.

[10] Su T, Mulla MS. Effects of Neem products containing azadirachtin on blood feeding, fecundity, and survivorship of *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). J. Vector. Ecol., 1999; 24: 202-15.

[11] Lucantoni L, Giusti F, Cristofaro M, Pasqualini L, Esposito F, et al. Effects ofneem extract on blood feeding, oviposition and oocyte ultrastructure in Anopheles stephensi Liston (Diptera: Culicidae). Tissue Cell, 2006; 38: 361-71.

[12] Mulla MS, Su T. Activity and biological effects of Neemproducts against arthropods of medical and veterinary importance. J. Am. Mosq. Control. Assoc., 1999; 15: 133-52.

[13] Boschitz C, Grunewald J. The effect of NeemAzal on *Aedesaegypti* (Diptera: Culicidae).Appl. Parasitol. 1994; 35: 251-6.

[14] Nathan SS, Kalaivani K, Murugan K. Effects of Neemlimonoids on the malaria vector Anopheles stephensi Liston (Diptera: Culicidae). Acta. Trop., 2005; 96: 47-55.

[15] World Health Organization. Instruction for determining thesusceptibility or resistance of mosquito larvae to insecticides.*Geneva:* WHO/VBC/81.807; 1981.

[16] Singh KRP, Patterson RS, LaBrecque GC, Razdan RK. Mass rearing of *Culex pipiensfatigans* Wied. J. Commun. Dis., 1975; 7 : 31-53.

[17] Brown MD, Thomas D, Watson K, Kay BH. Laboratory and field evaluation of efficacy of vectobac 12AS against *Culex sitiens* (Diptera: Culicidae) larvae. J. Am. Mosq. Control Assoc,1998; 1: 183-5.

[18] Abbott WS. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 1925; 18: 265-7.

[19] Finney DJ. Probit analysis, 3rd ed. Cambridge: Cambridge University Press; 1971.

[20] Sun C, Georghiou GP, Weiss K. Toxicity of *Bacillus thuringiensis* var *israelensis* to mosquito larvae variously resistant to conventional insecticides. Mosq. News, 1980; 40: 614-18.

[21] Aly C, Mulla MS, Xu B, Schnetter W. Rate of ingestion by mosquito larvae (Diptera: Culicidae) as a factor in the effectiveness of a bacterial stomach toxin. J. Med. Entomol., 1988; 25: 191-6.

[22] Aly C. Filtration rates of mosquito larvae in suspensions of latex microspheres and yeast cells. Entomol. Exp. Appl. 1988; 46: 55-61.

[23] Azmi MA, Naqvi SNH, Ahmad I, Tabassum R, Anbreen B. Toxicity of *Neem* leaves extracts (NLX) "compare with Malathion" (57 E.C.) against late 3rd instar larvae of *Culex fatigans* (Wild Strain) by WHO method. Trop. J. Zool., 1998; 22: 213-8.

[24] Zebitz CPW. Potential of Neem seed kernel extracts in mosquito control. Proceedings of the third International Neem Conference (1986 Nairobi, Kenya), 1987; pp. 555-73.

[25] El-Shazly MM, El-Sharnoubi ED. Toxicity of a Neem (*Azadirachta indica*) insecticide to certain aquatic organisms. J. Egypt. Soc. Parasitol. 2000; 30: 221-31.

[26] Caboni P, Cabras M, Angioni A, Russo M, Cabras P. Persistence of *azadirachtin* residues on olives after field treatment. J. Agric. Food Chem., 2002; 50: 3491-4.

[27] Caboni P, Sarais G, Angioni A, Garcia AJ, Lai F, Dedola F, et al. Residues and persistence of Neem formulations on strawberry after field treatment. J Agric Food Chem., 2006; 54: 10026-32.

[28] Govindarajan M, Jebanesan A, Pushpanathan T, Samidurai K. Studies on effect of Acalypha indica L. (Euphorbiaceae) leaf extracts on the malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). Parasitol Res., 2008; 103(3): 691-695.

How to cite this article:
Narendiran S, Janani D, Keerthana M, Nivethitha KS, Nirmala Devi S, Padmavathy S, Supraja TS, Sayeedur Rahman H, Velvizhi R, Swathi N, Yasaswini KG: Comparative Studies on *in-vitro* Phytochemicals Analysis and Larvicidal Efficacy of Medicinal Plant Extracts against *Culex quinquefasciatus*. Int. J. Life Sci. Scienti. Res., 2016; 2(6): 742-748. DOI:10.21276/ijlssr.2016.2.6.15

Source of Financial Support: Nil, **Conflict of interest:** Nil

