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Research Article

GC-MS analysis of *Eclipta prostrata* leaf extracts in relation to larvicidal activity against the mosquito *Aedes aegypti*

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Abstract

The mosquito is the principal vector of many of the vector borne diseases affecting human beings and animals. Several mosquito species vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue fever, dengue haemorrhagic 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. They even result in mutation of genes and these changes become prominent only after a few generations. Hence, the present study has been choosen to determine the photochemical screening, compound identification by GC-MS studyand larvicidal activity in various concentratingof *Eclipta prostrata* leaves extractagainst *Aedes aegypti*. The phytochemicals such as saponins, tannins, phenols, terpenoids, flavanoids, amino acids and protein, carbohydrates, glycosides were identified in both aqueous and methanol extracts, further revealed that *Eclipta prostrata* extract exhibited significantly highest larvicidal activity in 400 ppm at 24 hrs of methanol extract when compared to aqueous extract. The GC-MS analysis, showed 16 different compoundsand the compound isopropyl myristate, is found to be present in high area percentage.Further, the result of the currentinvestigation revealed that the methanol extract of *Eclipta prostrata* possess good larvicidal activity against *Aedes aegypti* with maximum larvicidal repellent and the numbers of the dead larvae were affected by increasing the concentrations of the plant extracts.

Introduction

India is one of the largest producers of medicinal herbs and is rightly called the botanical garden of the world as it is sitting on a gold mine of well-recorded and traditionally well practiced knowledge of herbal medicine. About 17,000 species of Indian flora about 7500 species of higher plants are reported to possess medicinal value and in other countries it is projected about 7% and 13%. In this regard India has a unique position in the world, where a number of recognized indigenous systems of medicine are available for the health care of people. No doubts that the herbal drugs are popular among rural and urban community of India. The demands for plant based medicines are increasing very fast in India. From this, we can say that search is goingon for more plantswhich can give therapeutic activity in treatment of various diseases [1].

Vector borne diseases are among the major causes of illness and death in many developing countries. Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world. WHO has declared the mosquitoes as “public enemy number one” [2]. Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people every year globally and 40,000,000 of the Indian population. They are nuisance to human beings and are the major vector for the transmission of life threatening diseases like malaria, dengue fever, yellow fever, chikunguniya fever, lymphatic filariasis and *Japaneses encephalitis* [3]. To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation is the

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application of synthetic insecticides such as organochlorine and organophosphate compounds. But mosquitoes have developed resistance against them. Though larvicides play a vital role in controlling mosquitoes in their breeding sites, these also show a negative impact in areas of beneficial and non-target organisms. Thus, synthetic insecticides have created several problems including the development of resistant insect strains, ecological imbalance and harm to mammals. Due to these drawbacks researchers are working hard to find biodegradable, target-specific and environmentally safe alternatives [4,5].

Mosquito control includes targeting the adult mosquito through spraying chemical insecticides or by killing the mosquito larvae before they emerge into adults via using synthetic larvicides or botanical extracts as an alternative larvicide. Synthetic larvicides also disrupt natural biological control systems that sometimes result into a wide spread development of resistance [6]. This phenomenon has triggered and urged the development of alternative using natural products. Current research trends use plant extract as alternative larvicides because they contain various phytochemicalsthat are specific in killing mosquito larvae without harming other organisms and the environment [7-10].

Dengue fever is caused by the arthropode borne flavivirus named dengue virus transmitted by the *Aedes aegypti* mosquito [11].Dengue fever is spread through the bite of an infected *Aedes aegypti* mosquito. The mosquito gets the virus by biting an infected person. The first symptom of the disease appears in about5-7 days of the infected mosquito bites a healthy person. 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 [12].Developing a safe and effective antiviral drug is difficult, because viruses use the host's cells to replicate. This makes it difficult to eradicate the virus without harming the host organism's cells [13]. It was clearly proved that crude or partially purified plant extracts are less expensive and highly efficacious for control of mosquitoes [14].The plant extract are eco-friendly and are not toxic to vertebrates [15].

Eclipata prostrata Linn is a common plant and abundantly grows throughout India up to 6000 ft heights of hills.It is commonly known as Trailing Eclipta in English, Bhanagra in Hindi and Kayyanthara in Tamil. It is an ereact or prostrate annual herb and the leaves are opposite, sessile and lanceolate.The leaves are densely arrangedon both sides of the stem and rooting at the nodes and the flower-heads are white [16]andis popularly known as false daisyBhingaraj.They has great traditional reputations of being used by the rural people of Tamil Nadu for several human illnesses like kidney and liver weakness, inflammatory conditions, opthalamic and digestive disorders. It is also regarded as the best haematinic, diuretic and anthelmintic [17].

Plant may be a source of alternative agents for control of mosquitoes, because they are rich in bioactive chemicals, are active against limited number of species including specific target-insects and are biodegradable, because mosquitoes develop genetic resistance to synthetic insecticides and even to biopesticides. Hence, the present study was undertaken to evaluate mosquito larvicidal potentiality of aqueous and methanol leaf extracts of *Eclipta prostrata* against the fourth-instar larvae of a *Aedes aegypti* and to the screen the phytochemical present in the leave extracts and to identify the active compound present in the methanol extract of *Eclipta prostrata* leaves.

Methodology Collection and extraction of plant material

The fresh leaves of *Eclipta prostrata* plant were collected from in and around Thanjavur District, Tamilnadu, India.

Preparation of extract

The plant material was shade dried for three days. After drying, plant material is powdered with the help of mixer grinder. Twenty gram of powdered plant material was mixed with 100ml solvent like aqueous and methanol. The extracts prepared in succession from powdered leaf material by soxhlet method. The collected extracts stored in a vial for further studies.

Phytochemical Screening

Different qualitative chemical tests can be performed for its chemical composition using standard procedures described by Harborne [18], Trease and Evans [19] and Sofowara[20].

Test organism (Mosquitoes) - sample collection

Eggs of *Aedes aegypti* is obtained from Centre for Research in Medical Entomology (CRME), Madurai, Tamilnadu, India. Test organisms were reared in trays containing tap water and maintained at 28±2**°** c in the laboratory. The eggs were hatched out into first instar larvae and they were fed with yeast powder and glucose. On the third day the first in star molted into second instar. On the fifth day it turned to third instar and the fourth instar was molted on the seventh day. The larvae were fed with larval food (dog biscuit and glucose in the ratio 3:1).The fourth in star larvae of *Aedes aegypti* was used in the present study (Fig.1)

Larvicidal bioassay

The larvicidal bioassay was carried out by using standard WHO protocols [21].The tests were conducted in 5 liter tray with 2 liter of tap water in a series.The test concentrations were made with both the aqueous and methanol extracts of *Eclipta prostrata* respectively.From the stock solution, different concentrations were prepared as 100 ppm, 200 ppm, 300 ppm and 400 ppm.The immature mosquitoes particularly fourth instar larvae were used for the present study and they fed with yeast powder and glucose on the water surface.10 larvae per concentration were exposed to each dose, the number of dead larvae was recorded for every 12 hours for two days post treatment at room temperature.The larvae considered to dead when they showed no sign of movement (or) immobile and unable to reach the water surface.They were disposed with the proceed using a needle and mortality

data was analyzed by using Abbott's method [22]. The experiment was repeated for six times.

GC-MS analysis

GC-MS analysis was carried out on a Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CTO6859, and USA) which includes a Perkin Elmer Auto sampler XLGC. The column used was Perkin Elmer Elite - 5 capillary column measuring 30m × 0.25mm with a film thickness of 0.25mm composed of 95% Dimethyl polysiloxane. The carrier gas used was Helium at a flow rate of 0.5ml/min. 1µl sample injection volume was utilized. The inlet temperature was maintained as 250°C. The oven temperature was programmed initially at 110°C for 4 min, then an increase to 240°C. And then programmed to increase to 280°C at a rate of 20°C ending with a 5 min. Total run time was 90 min. The MS transfer line was maintained at a temperature of 200°c. The source temperature was maintained at 180°c. GCMS was analyzed using electron impact ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library. Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software.

Statistical analysis

The results obtained in the present investigation were analysed statistically using standard deviation (SD) and Duncan's multiple range test (DMRT) [23].

The data were compiled using statistical package for social science program (SPSS 16th version) and subjected to analysis of variance (ANOVA) with post hoc Duncan's test comparison [24] (oneway), $P<0.05$ was considered as significance.

Results

The present investigation deals with the screening of phytochemicals present in the aqueous and methanol extracts of *Eclipta prostrata* leaves.Test leaves was screened for larvicidal activities against *Aedes aegypti* and the compounds present in the methanol extract were also identified using GC – MS.

The phytochemical compounds of *Eclipta prostrata* qualitative analyzed, further the present study showed the presence of phytochemical compounds such as saponins, tannins, phenols, terpenoids, flavanoids, aminoacid and proteins, carbohydrates, glycosides were present in both aqueous and methanol extract. The compounds phytobatannins, volatile oils were exclusive present in methanol extracts and were absent in aqueous extracts. The compounds steroids were absent in both aqueous and methanol extracts (Table – I).

Table 1: Preliminary phytochemical studies on various extracts of *Eclipta prostrate* leaves powder

S. No.	Phytochemical tests	Aqueous	Methanol
1	Saponins	+	+
2	Tannins	+	+
3	Phenols	+	+
4	Steroids	-	-
5	Terpenoids	+	+
6	Flavanoids	+	+
7	Amino acid and proteins	+	+
8	Carbohydrates	+	+
9	Phytobatannins	-	+
10	Volatile oils	-	+
11	Hydrolysable Tannins	+	-
12	Glycosides	+	+

'(+)' Present '(-)' Absent

The larvicidal activity of *Eclipta prostrata* aqueous extracts showed significantly high effect in 400ppm (8.50 ± 0.34) concentration at 48 hrs against *Aedes aegypti* but it does not reaches the 100% mortality in aqueous extract.The 100 ppm concentration showed significantly minimum mortality when compared to other concentrations ie., no effect on *Aedes aegypti*.

The methanol extract of *Eclipta prostrata* showed significantly high larvicidal effect at 400 ppm (10.00 ± 0.33) 24 hrs treatment group against *Aedes aegypti*. The larvicidal activity was increase gradually as the concentration of the extract increased. It is noticed that the concentration of the extract increased, the mortality rate of the larvae was also increased. Over all aqueous and methanol extracts larval mortality of methanol extract *Eclipta prostrate* showed 100% mortality at 400 ppm 24 hrs treatment group when compared to aqueous against *Aedes aegypti*. To conclude the results, the present study revealed that the methanol extract of *Eclipta prostrata* showed maximum activity against *Aedes aegypti* (Table -2).

Table 2: Larvicidal activity of *Aedes aegypti* against *Eclipta prostrata* plant extracts

Concentration of plant extracts (ppm)	Time interval (Hours)	*Eclipta prostrata*	
		Aqueous	Methanol
Control	12	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}
	24	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}
	36	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}
	48	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}
100	12	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}
	24	0.00 ± 0.00^{g}	2.00 ± 0.25^{g}

	36	2.33 ± 0.33^{f}	4.66 ± 0.33^{e}
	48	4.83 ± 0.30^{bc}	6.33 ± 0.33^{de}
200	12	0.00 ± 0.00^{g}	4.33 ± 0.33^{f}
	24	2.33 ± 0.33^{f}	5.33 ± 0.21^{de}
	36	4.83 ± 0.30^{bc}	7.33 ± 0.21^{c}
	48	6.33 ± 0.5^{a}	8.33 ± 0.21^{b}
300	12	0.00 ± 0.00^{g}	5.50 ± 0.21^{e}
	24	2.83 ± 0.30^{ef}	6.33 ± 0.49^{de}
	36	5.33 ± 0.42^{cd}	7.00 ± 0.36^{d}
	48	8.33 ± 0.51^{a}	8.50 ± 0.22^{c}
400	12	3.50 ± 0.34^{de}	7.16 ± 0.30^{c}
	24	5.66 ± 0.42^{b}	10.00 ± 0.33^{a}
	36	6.50 ± 0.34^{b}	00.00 ± 0.00^{h}
	48	8.50 ± 0.34^{a}	00.00 ± 00.00^{h}

Values are expressed in Mean ±SE. Different superscript alphabets within the column indicate statistical significant difference in larvicidal activity between concentrations at P<0.05 level.

Phyto components present in the methanol extract *Eclipta prostrata* leaves were identified using GC-MS.Nearly 16 compound were identified and the compounds were fell between the retention time 2.94 min to 13.38 min.The compounds are octanoic acid, 8-hydroxy-metyl…(2.94), acetylacetone, monoxime (3.85), cyclobutanone, 2-methyl-2-oxiranyl- (4.34), 4-methyl-1-hepten- 4-ol acetate (4.85), 3-nonynoic acid (6.80), dodecanoic acid, methyl ester (7.10), acetonitrile, 2,2'-iminobis (7.50), cyclobutanone,2-methyl-2-oxiranyl- (8.25), carbonic acid, ethyl octadecyle (8.50), methyl teradecanoate (9.32), benzisoxazole -2-acetic acid, hydroxyl (9.80), isopropyl myristate (10.37), 2-propenoic acid, 3-(3-fluorophenol) (10.86), cyclopentaneundecanoic acid, methyl (11.38), n- hexadecanoic acid (11.70), 9-octadecanoic acid (13.30).

Among 16 compounds the compound isopropyl myristate ($C_{17}H_{34}O_2$, 270.45) fell at retention time 10.37min is the present in high area percentage (56.97%) when compared to other compounds. Next to this, the compound9-octadecanoic acid($C_{18}H_{34}O_2$, 283.46) is present in high area percentage (24.21) at retention time 13.30 min, followed by, n-hexadecanoic acid ($C_{16}H_{32}O_2$, 256.43) which is present in high area percentage (7.21%) at the retention time 11.70 min. The compounds cyclobutanone, 2-methyl-2-oxiranyl ($C_{10}H_{18}O_3$, 186.25) and Carbonoic acid, ethyl octadecyle ($C_{21}H_{42}O_3$, 342.55) where present in low are percentage (0.44, 0.55%) at retention time 8.25 and 8.80 respectively(Table 3, Fig. 1).

Table 3: Phyto-components identified in the methanol extract of *Eclipta prostrata* leaves using GC-MS

Peak No.	Compounds Name	Mol. Formula	R. Time	Mol. Weight	Area % S
I	Octanoic acid, 8-hydroxy-metyl	$C_8H_1O_2$	2.94	144.21	2.29
II	Acetylacetone, monoxime	$C5H_8O_2$	3.85	100.13	0.94
III	Cyclobutanone, 2-methyl-2-oxiranyl	$C_7H_{10}O_2$	4.34	126.15	0.77
IV	4- Methyl-1-hepten- 4-ol acetate	$C_9H_{16}O$	4.85	140.22	0.70
V	3-Nonynoic acid	$C_3H_6O_2S$	6.80	106.13	0.44
VI	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	7.10	200.31	0.86
VII	Acetonitrile, 2,2'-iminobis	$C_{18}H_{34}O_2$	7.50	95.11	1.30
VIII	Cyclobutanone,2-methyl-2-oxiranyl	$C_{10}H_{18}O_3$	8.25	186.25	0.44
IX	Carbonic acid, ethyl octadecyle	$C_{21}H_{42}O_3$	8.80	342.55	0.55
1X	Methyl teradecanoate	$C_{15}H_{30}O_2$	9.32	242.40	1.13
XI	Benzisoxazole -2-acetic acid, hydroxyl	$C_9H_7NO_3$	9.80	177.15	0.48
XII	Isopropyl myristate	$C_{17}H_{34}O_2$	10.37	270.45	56.97
XIII	2-Propenoic acid, 3-(3-fluorophenol)	$C_3H_4O_3$	10.86	88.06	1.08
XIV	Cyclopentaneundecanoic acid, methyl	$C_{16}H_{30}O_2$	11.38	254.41	0.62
XV	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	11.70	256.43	7.21
XIV	9-Octadecanoic acid	$C_{18}H_{34}O_2$	13.38	282.46	24.21

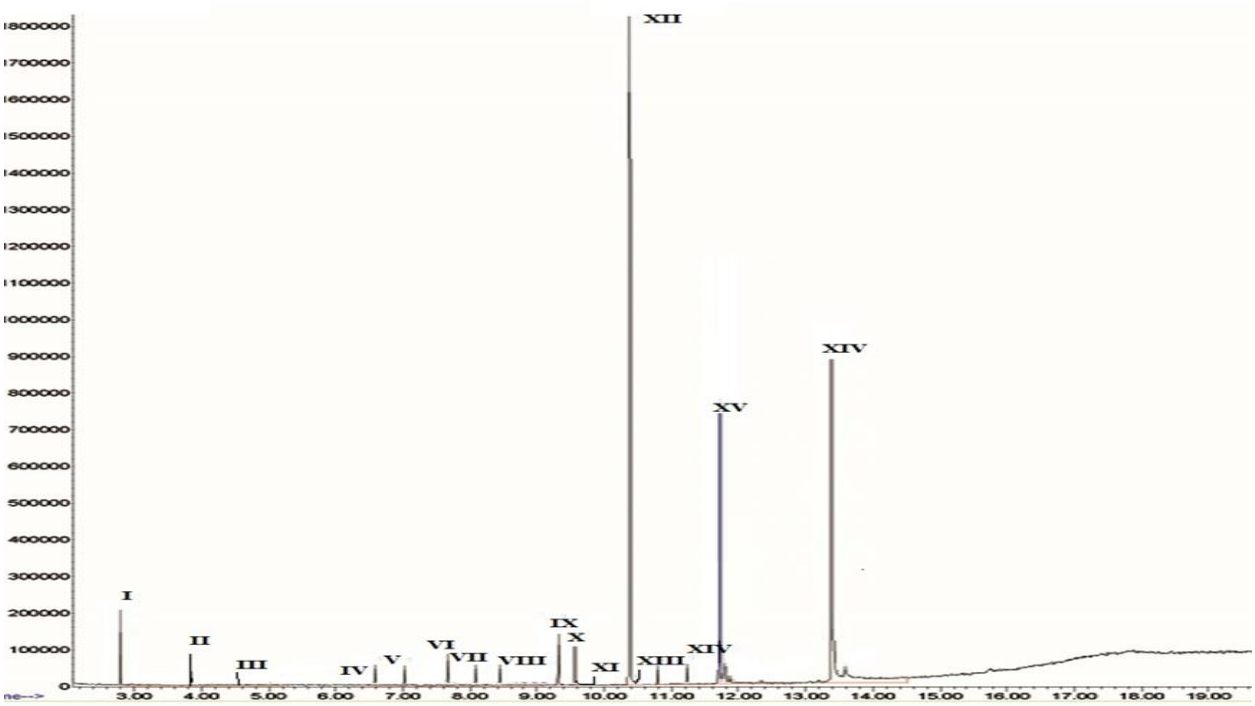

Fig. 1:Identification of active compounds in the methanol extract of *Eclipta prostrata* leaves using GC- MS

Discussion

Globally there has been a conscientious effort by scientists to overcome these problems and great emphasis has been placed recently on green chemistry for mosquito control using natural plant products. It is well known that natural products derived from plants are effective, safe and extensively used as biologically active compounds particularly in the area of infectious diseases [25]**.** Several studies have focused on the plant products as effective insecticides and larvicides for controlling different species of mosquitoes [26]. Natural products from plants are alternative sources of insect control agents since they contain a range of bioactive chemicals, which are selective and do not harm non-target organisms and the environment [27,28].Plants have formed the basis of natural pesticides that make excellent leads for new pesticide development [29].During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic and chemical insecticides.

In our present phyto components study,saponins, tannins, phenols, terpenoids, flavanoids, amino acid and proteins, carbohydrates, glycosides were present in both aqueous and methanol extracts of *Eclipta prostrata.* The reported phytochemicals derived from plant sources can act as larvicide, insect growth regulators, and repellent and ovipositor attractant and have different activities observed by many researchers [30-32]. However, insecticides of plant origin have been extensively used on agricultural pests and to a very limited extent, against insect vectors of public health importance. Larvicidal activities of the plant extracts vary according to the plant species, the parts of the plant, the geographical location where the plants were grown and the application method. 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 [33].

Saponins, which is one of the active constituents involved in plant disease resistance because of its antimicrobial and antilarvicidal activity [34]. Traditionally, saponins are subdivided into triterpenoid and steroid glycoside, tannins are phenolic compound which act primary antioxidants or free radical scavenger [35].

Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, and repellent and ovipositor attractant and have different activities observed by many researchers [36-37]. However, insecticides of plant origin have been extensively used on agricultural pests and to a very limited extent, against insect vectors of public health importance. Larvicidal activities of the plant extracts vary according to the plant species, the parts of the plant, the geographical location where the plants were grown and the application method. 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 [38].

Several secondary metabolites such as steroids [30-41]phenolicsessential oils [42] were reported to have a

remarkable mosquito larvicidal activity. Plant-derived substances have recently become of great interest due to their multipleapplication [43]. The medicinal plants are the richest bioresource of drugs of traditional systems of medicine [44].Even today plant materials continue toplay a major role in primary health care as therapeutic remedies in many developing countries [45]. Botanicals are basically secondary metabolites that serve as a means of defence mechanism of the plants to withstand the continuous selection pressure from herbivore predators and other environmental disturbing organism

Mosquito borne diseases are one of the most public health problems in the developing countries. It can be controlled by preventing mosquito bite using repellent, causing larval mortality and killing mosquitos. The effects of various extracts were studied in a dose dependent manner. Senthilnathan, [46] observed that higher larvicidal effect of *Eucalyptus tereticornis* oil with increased doses on *Anopheles stephensi*, which was correlated with our findings, that methanol extracts shows maximum mortality when compare to aqueous.The plant *M. Hortensis* has apotent mosquito larvicidal property a pertaining to its effect on growth and development of mosquitoes. This can also be used for control of mosquito breeding [47,48]. They also offer this as a safer alternative to synthetic chemicals and can be obtained by individuals and communities easily at a very low cost. However, toxicity tests of the active plants need to be done to ascertain their safety in administration [49,50].

Recently, bio-pesticides with plant origins are given for use against several insect species, especially disease transmitting vectors, based on the fact that compounds of plant origin are safer to use, without phototoxic properties and leave no scum in the environment [51]. Chapagain and Wiesman, [52] reported that saponin extracted from the fruit of *Balanitesaegyptiaca* showed 100% larvicidal activity against *Aedes aegypti* mosquito larvae. They have suggested that the saponin molecules interact with the cuticle membrane of the larvae, ultimately disarranging the membrane could be the most probable reason for the larval death. The deficiency of dissolved oxygen and active presence of the antioxidant saponin molecule might be the reason for larval death. However, much study is required to find out the mechanism by which saponin kills the larvae. A commercial saponin mixture extracted from *Q. saponaria* showed increasing toxicity (100% larval mortality) in *Aedes aegypti* and *Culexpipiens* when both saponin concentration and the duration of the experiment were increased [53]. This result support our present study, that methanolic extract of *Eclipta prostrata* show significantly high mortality *ie.*,100% in 400 ppm at 24 hrs of treatment.The result of the currentinvestigation revealed that the methanol extract of *Eclipta prostrata* possess good larvicidal activity against *Aedes aegypti* with maximum larvicidal repellent and the numbers of the dead larvae were affected by increasing the concentrations of the plant extracts.

Preventive measures should be taken before spreading of any kind of disease including dengue fever. Hence, the study concluded that *Eclipta prostrata* leaf methanol extract effective has larvicidalproperty against *Aedes aegypti*. Further, research can be developed by examining the content of identified compounds in*Eclipta prostrata*extract which may beresponsible for larvicidal activityand can be tested their effects on the isolation lab trails.

References

1. PatwardhanMendas P, Dixnn RA. Plant metabolomics large scatephytochemistry in the functional genomics era, Phy. Chlm. 2004; 62:817-836.
2. WHO, World malaria situation in 1994.Part I. Population at risk, Weekly Epidem. Rec. 1997; 72:36: 269-274.
3. Anupam Ghosh, Nandita Chowdhury, Goutam Chandra. Plant extracts as potential mosquito larvicides. Indian J. Med. Res. 2012; 135:581-598.
4. Luz Stella Nerio, Jesus Olivero-Verbel, Elena Stashenko. Repellent activity of essential oils,A review Bioresource Technology, 2010; 101:372– 378.
5. Sritabutra D, Soonwera M, Sirirat S, Poungjai S. Evaluation of herbal essential oil as repellents against Aedes aegypti(L.) and Anophelesdiruspeytonand Harrion, Asian Pacific J. Trop. Biomed. 2011; 1:1:124-128.
6. Tiwari M, Naik SN, Tewary DK, Mittal PK, Yadav S. Chemical composition and larvicidal activities of the essential oil of Zanthoxylumarmatumagainst three mosquito vectors, J. Vector Borne Diseases.2007; 44:3:198.
7. Arnason JT, Philogene BJR, Morand P. Insecticides of plant origin, Amer. Chem. Society, 1989, 213-217.
8. Hedlin PA, Holingworth RM, Masler EP, Miyamoto J, Thopson DG. Phytochemicals for pest control. ACS SympSer No. 658. Washington DC: American Chemical Society. 1997, 372.
9. Tiwari M, Naik SN, Tewary DK, Mittal PK, Yadav S. Chemical composition and larvicidal activities of the essential oil of Zanthoxylumarmatumagainst three mosquito vectors, .J. Vector Borne Diseases.2007; 44:3:198.
10. Almeida APG. Mosquitoes (Diptera:Culicidae) and their medical importance for Portugal.Review.Challenges for the XXI century, Acta. Medica. Portuguesa, 2011; 24: 961-974.
11. Talarico LB, Zibetti RG, Noseda MD, Duarte MER,Damonte EB. An algal-derived DL-galactan hybrid is an efficient preventing agent for in vitro Dengue virus infection. Plant. Med., 2007; 73:1464-1468.
12. WHO, 2012, World Health Organization: Dengue and severe dengue (factsheet no. 117, revised January 2012).
13. Golan DE, Tashjian AH, Armstrong EJ, Amstrong AW. Principles of pharmacology: The pathophysiologic basic of drug therapy. Philadelphia: Lippincott Williams and Wilkins, Second edition, 2008.
14. Jang YS, Kim MK,Ahnys Lee HS. Larvicidal activity of Brazilian plantagainst Aedes aegypti and Culex pipiens

(Diptera: culicidae), Agrichem. Bio. Technol., 2000; 24: 131-134.

15. Sharook AL, Balan ZK, Jing Y, Rembnold H. Insect growth inhibitor from two tropical meliaceae, effect of crude seeds extracts on mosquito larvae, J. App. Ento., 1991; 111:425-430.

16. Asolkar LV, Kakkar KK, Charkra OJ. Second Supplement to Glossary of Indian Medicinal Plants with Active Principles Part –I,NISC, CSIR, New Delhi, India, 1992; 265-266.

17. Prajapat MS, Patel JB, Modi MB, Shah E.Leucasaspera: A review Pharmacogn. 2010; 4:85-87.

18. Harborne JB. Phytochemical methods. A guide to modern techniques of plant analysis. London: Chapman and Hall, 1984; 49-188.

19. Trease GE, Evans WC. Pharmacology (11^{th}) Braille tirida, Canada Macmillan publishers, Canada. 1989, 257.

20. Sofowara A. Medicinal plants and traditional medicine in Africa, Spectrum Books Ltd, Ibadan, Nigeria. 1993, 289

21. WHO., World Health Organization guidelines for laboratory and field testing of mosquito larvicides. 2005.

22. Abbott WS. A method of computing the effectiveness of an insecticide, J. Economic Entomology, 1925; 18:265–267

23. Zar JH. In Biostatistical Analysis, Englewood cliffs, N.J.prentive hall, Inc. 1984; 3:123- 129.

24. Duncan DB. Multiple range and multiple test, Biometrics, 1995; 11:1-42.

25. Cragg GM, Newman DJ, Snader KM. Natural productsin drug discovery and development, J. Nat. Prod. 1997; 60(1):52-60.

26. Samuel T, Ravindran KJ, Arivoli S. Screening of twenty five plant extracts for larvicidal activity against Culex quinquefasciatus Say (Diptera: Culicidae). Asian Pacific J. Trop. Biomed. 2012; 2:1130-1134.

27. Arnason JT, Philogene BJR, Morand P. Insecticides of plant origin, Amer. Chem. Society, 1989, 213-217.

28. Hedlin PA, Holingworth RM, Masler EP, Miyamoto J,Thopson DG. Phytochemicals for pest control. ACS SympSer No. 658. Washington DC: American Chemical Society. 1997, 372.

29. Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery, Natural Product Research. 2000; 17:215–34.

30. Babu R, Murugan K. Interactive effect of neem seed kernel and neem gum extracts on the control of Culex quinquefasciatus Say, Neem Newsletters. 1998; 15:2:9-11.

31. Venkatachalam MR, Jebanesan A. Repellent activity of FerroniaelephantumCorr. (Rutaceae) leaf extract against Aedes aegypti. Bioresour. Technol. 2001a; 76 :287–288.

32. Venkatachalam MR, Jebanesan A. Larvicidal activity of HydrocotylejavanicaThunb.(Apiaceae) extract against Culex quinquefasciatus. J. Exp. Zool, 2001b: 4:99–101.

33. Das NG, Goswami D, Rabha B. Preliminary evaluation of mosquito larvicidal efficacy of plant extracts, J. Vector Borne Diseases, 2007; 44:145-148.

34. Barile E, Bonanomi G, Antignani V, Zolfaghari B, EbrahimSajjadi S, Scala F, Lanzotti V. Saponin from Allium minutiflorum with antifungal activity. Phytochemistry. 2007; 68:596-603.

35. Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC,Atangbayila TO. Phytochemical screening and antioxidant activities of selected medicinal plants used for malaria therapy in Southwestern Nigeria,Trop. J. Pharm. Res. 2008; 7:1019-1024.

36. Babu R, Murugan K. Interactive effect of neem seed kernel and neem gum extracts on the control of Culex quinquefasciatus Say, Neem Newsletters, 1998; 15(2):9-11.

37. Venkatachalam MR, Jebanesan A. Larvicidal activity of HydrocotylejavanicaThunb(Apiaceae) extract against Culex quinquefasciatus. J. Exp. Zool. 2001; 4(1):99–101.

38. Das NG, Goswami D, Rabha B. Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. J. Vector Borne Diseases. 2007; 44:145-148.

39. Ghosh A, Chowdhur YN, Chandra G. Plant extracts as potential mosquito larvicides. Indian J. Med. Res. 2012; 135:581-598.

40. ChowdhuryRRC, Singh AP, Ghosal S, Prasad BNK, Srivastava V, Tandon JS. Possible mechanism of hepato protective activity of Azadrachia indica left extract part II, J. Ethanopharmcol. 2008; 89:217-219.

41. Rahuman AA, Venkatesan P, Gopalakrishnan G. Mosquito larvicidal activity of oleic and linoleic acids isolated from Citrullus colocynthis (Linn.)Schrad, Parasitol Res. 2008; 103:1383–1390.

42. Amer A,Mehlhor H. Larvicidal effects of various essential oils against Aedes, Anopheles and Culex larvae, Parasitol. Res. 2006; 99:466-472.

43. Kavitha S, Rajeshwari S, Hasna A, Rajiv P. Pharmacognostical and phytochemical screening of Asystasiagantetica (Chinese violet),2013.

44. Sukanya SL, Sudisha J, Hariprasad P, Niranjana SR, Prakash HP, Fathima SK. Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria, Afr. J. Biotechnol. 2009; 7:33-43.

45. Augusti G, Squarzoni A. 'L' accreditamentodeglistudi di Ingegneria.IIprogetto EUR-ACE, Quadernide llaconferenzadeipreidibdellefacolta di Ingegneria No. 4; CUES, Salerno. 2008

46. Senthilnathan S. The use of Eucalyptustereticornis Sm. (Myrtaceae) oil (leaf extract) as anatural larvicidal agent against the malaria vectorAnopheles stephensi Liston (Diptera: Culicidae),Biosource Tech. 2007; 98:1856-1860.

47. Barnard DR, Dexue RD. Laboratory evaluation of mosquito repellents against Aedes albopictus, Culex

nigripalpus, and Ochlerotatustriseriatus(Diptera: Culicidae), J. Med. Entomol, 2004: 41:726-730.

48. Trongtokit Y, Rongsriyam Y, Komalamisra N, Apiwathnasorn C. Comparative repellency of 38 essential oils against mosquito bites, Phytother Res. 2005; 19:303-309.

49. Robert LL, Hallan JA, Seeley DC, Roberts LW, Wirtz RA. Comparative sensitivities of four Anopheles (Diptera: Culicidae) to five repellents. J. Med. Ent. 1991; 28:417-420.

50. Rutledge LC, Moussa MA, Lowe CA, Sofield RK. Comparative sensitivity of mosquito species and strains to the repellent diethyl toluamide. J Med.Ent. 1978; 14:536-541.

51. Schmutterer H. Properties and potential of natural pesticides from the neem tree, Azadirachta indica. Ann. Rev. Ent.1990; 35:271-297.

52. Chapagain B, Wiesman Z. Larvicidal effects of aqueous extracts of Balanitesaegyptiaca (desert date) against the larvae of Culex pipiens mosquitoes, Afr.J. Biotechnol, 2005; 4:11:1351 -1354.

53. Pelah D, Abramovich Z, Markus A, Wiesman Z. The use of commercial psaponin from Quillajasaponoaria bark as a natural larvicidal agent against Aedes aegypti and Culex pipiens. J. Ethnopharmacol, 2005; 81 (2):407-409

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