

**SCREENING OF BIOLOGICAL LARVICIDAL  
ACTIVITY GUIDED FRACTIONS OF BEST SELECTED  
COMMERCIALY AVAILABLE PLANTS, AGAINST  
*Aedes*, *Culex* AND *Anopheles* VECTOR SPECIES  
OF MOSQUITOES FOR HERBAL FORMULATION**

Thesis Submitted for the Award of the Degree of

**DOCTOR OF PHILOSOPHY**  
in  
**Zoology**

By

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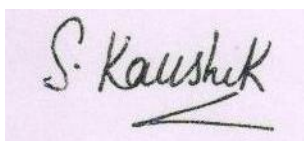
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**LOVELY PROFESSIONAL UNIVERSITY, PUNJAB**

**2024**

## DECLARATION

I, hereby declared that the presented work in the thesis entitled “**Screening of biological larvicidal activity guided fractions of best selected commercially available plants, against *Aedes*, *Culex* and *Anopheles* vector species of mosquitoes for herbal formulation**” in fulfilment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision Dr Neeta Raj Sharma, working as Professor and Dean, in the Bioengineering and Biosciences of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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**(Signature of Scholar)**

Name of the scholar: Shweta Kaushik

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## CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled, **“Screening of biological larvicidal activity guided fractions of best selected commercially available plants, against *Aedes*, *Culex* and *Anopheles* vector species of mosquitoes for herbal formulation”** submitted in fulfilment of the requirement for the reward of degree **Doctor of Philosophy (Ph.D.)** in the school of Biotechnology and Biosciences, is a research work carried out by Shweta Kaushik, registration no. 41500127 is bonafide record of her original work carried out under my supervision and that no part of this thesis has been submitted for any other degree.

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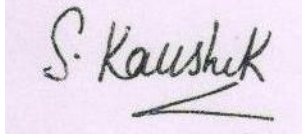
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A handwritten signature in black ink on a light purple rectangular background. The signature reads "S. Kaushik" in a cursive style, with a horizontal line underneath the name.

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## TABLE OF CONTENTS

<b>INDEX</b>		<b>PAGE NO.</b>
<b>Chapter 1</b>	<b>INTRODUCTION</b>	<b>01-04</b>
<b>Chapter 2</b>	<b>REVIEW OF LITERATURE</b>	<b>05-23</b>
<b>Chapter 3</b>	<b>MATERIALS AND METHODS</b>	<b>24-38</b>
<b>Chapter 4</b>	<b>RESULTS AND DISCUSSION</b>	<b>39-141</b>
<b>Chapter 5</b>	<b>SUMMARY AND CONCLUSION</b>	<b>142-144</b>
<b>Chapter 6</b>	<b>BIBLIOGRAPHY</b>	<b>145-159</b>
<b>Chapter 7</b>	<b>ACRONYMS AND ABBREVIATIONS</b>	<b>160-161</b>
<b>Chapter 8</b>	<b>APPENDICES</b>	<b>162-169</b>

## LIST OF TABLES

S.No.	Table No.	Title	Page No.
1	2.1	National Vector Borne Disease Control Programme, the status of cases due to vector borne diseases in India	06
2	2.2	Taxonomic hierarchy of selected plants	10
3	3.1	List of phytoconstituents of the selected plants	25
5	3.2	Details of the proteins retrieved from <i>Ae aegypti</i> , <i>Culex quinquefasciatus</i> , <i>Anopheles stephensi</i>	28
6	3.3	Detail of plants used in the study	34
7	4.1	Tabular display of docking score of the <i>Aedes</i> larval essential proteins with phyto constituents	42
8	4.2	Tabular display of docking score of the <i>Culex</i> larval essential proteins with phyto constituents	81
9	4.3	Tabular display of docking score of the <i>Anopheles</i> larval essential proteins with phyto constituents	90
10	4.4	Larval toxicity effect of various solvent extracts of <i>Calotropis procera</i> leaves against dengue vector, <i>Ae. aegypti</i>	99
11	4.5	One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of <i>Calotropis procera</i> against <i>Ae. aegypti</i> .	100
12	4.6	One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of <i>Calotropis procera</i> against	101

		<i>Ae. aegypti.</i>	
13	4.7	One way ANOVA analysis between the mortality rate of different replicates and the concentration of acetone extract of <i>Calotropis procera</i> against <i>Ae. aegypti.</i>	101
14	4.8	One way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of <i>Calotropis procera</i> against <i>Ae. aegypti.</i>	102
15	4.9	Larval toxicity effect of various solvent extracts of <i>Calotropis procera</i> leaves against filaria vector, <i>Cx. quinquefasciatus</i>	103
16	4.10	One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of <i>Calotropis procera</i> against <i>Cx. quinquefasciatus.</i>	104
17	4.11	One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of <i>Calotropis procera</i> against <i>Cx. quinquefasciatus.</i>	105
18	4.12	One way ANOVA analysis between the mortality rate of different replicates and the concentration of acetone extract of <i>Calotropis procera</i> against <i>Cx. quinquefasciatus.</i>	105
19	4.13	One-way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of <i>Calotropis procera</i> against <i>Cx. quinquefasciatus.</i>	106
20	4.14	Larval toxicity effect of various solvent extracts	107



		of <i>Calotropis procera</i> leaves against malaria vector, <i>An. stephensi</i>	
21	4.15	One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of <i>Calotropis procera</i> against <i>An. stephensi</i>	108
22	4.16	One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of <i>Calotropis procera</i> against <i>An. stephensi</i>	109
23	4.17	One way ANOVA analysis between the mortality rate of different replicates and the concentration of acetone extract of <i>Calotropis procera</i> against <i>An. stephensi</i>	109
24	4.18	One-way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of <i>Calotropis procera</i> against <i>An. stephensi</i>	110
25	4.19	Larval toxicity effect of various solvent extracts of <i>Aegle marmelos</i> leaves against dengue vector, <i>Ae. aegypti</i> .	112
26	4.20	One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of <i>Aegle marmelos</i> against <i>Ae. aegypti</i> .	113
27	4.21	One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of <i>Aegle marmelos</i> against <i>Ae. aegypti</i> .	113
28	4.22	One way ANOVA analysis between the mortality rate of different replicates and the concentration	114

		of acetone extract of <i>Aegle marmelos</i> against <i>Ae. aegypti</i>	
29	4.23	One way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of <i>Aegle marmelos</i> against <i>Ae. aegypti</i> .	114
30	4.24	Larval toxicity effect of various solvent extracts of <i>Aegle marmelos</i> leaves against filaria vector, <i>Cx. quinquefasciatus</i>	116
31	4.25	One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of <i>Aegle marmelos</i> against <i>Cx. quinquefasciatus</i> .	117
32	4.26	One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of <i>Aegle marmelos</i> against <i>Cx. quinquefasciatus</i>	117
33	4.27	One way ANOVA analysis between the mortality rate of different replicates and the concentration of acetone extract of <i>Aegle marmelos</i> against <i>Cx. quinquefasciatus</i> .	118
34	4.28	One way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of <i>Aegle marmelos</i> against <i>Cx. quinquefasciatus</i> .	118
35	4.29	Larval toxicity effect of different solvents of <i>Aegle marmelos</i> leaves against malaria vector, <i>An. stephensi</i>	120
36	4.30	One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of <i>Aegle marmelos</i> against <i>An.</i>	121

		<i>stephensi</i>	
37	4.31	One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of <i>Aegle marmelos</i> against <i>An. stephensi</i>	121
38	4.32	One way ANOVA analysis between the mortality rate of different replicates and the concentration of acetone extract of <i>Aegle marmelos</i> against <i>An. stephensi</i>	122
39	4.33	One way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of <i>Aegle marmelos</i> against <i>An. stephensi</i>	122
40	4.34	Larval toxicity effect of different solvents of <i>Agave americana</i> leaves against dengue vector, <i>Ae. aegypti</i> .	124
41	4.35	One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of <i>Agave americana</i> against <i>Ae. aegypti</i>	125
42	4.36	One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of <i>Agave americana</i> against <i>Ae. aegypti</i> .	126
43	4.37	One way ANOVA analysis between the mortality rate of different replicates and the concentration of acetone extract of <i>Agave americana</i> against <i>Ae. aegypti</i>	126
44	4.38	One way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of <i>Agave americana</i> against <i>Ae. aegypti</i>	127

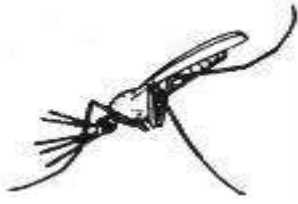
45	4.39	Larval toxicity effect of various solvent extracts of <i>Agave americana</i> leaves against filaria vector, <i>Cx. quinquefasciatus</i> .	128
46	4.40	One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of <i>Agave americana</i> against <i>Cx. quinquefasciatus</i> .	129
47	4.41	One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of <i>Agave americana</i> against <i>Cx. quinquefasciatus</i>	130
48	4.42	One way ANOVA analysis between the mortality rate of different replicates and the concentration of acetone extract of <i>Agave americana</i> against <i>Cx. quinquefasciatus</i> .	130
49	4.43	One way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of <i>Agave americana</i> against <i>Cx. quinquefasciatus</i>	131
50	4.44	Larval toxicity effect of various solvent extracts of <i>Agave americana</i> leaves against malaria vector, <i>An. stephensi</i> .	132
51	4.45	One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of <i>Agave americana</i> against <i>An. stephensi</i> .	133
52	4.46	One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of <i>Agave americana</i> against <i>An. stephensi</i> .	134
53	4.47	One way ANOVA analysis between the mortality	134

		rate of different replicates and the concentration of acetone extract of <i>Agave americana</i> against <i>An. stephensi</i> .	
54	4.48	One way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of <i>Agave americana</i> against <i>An. stephensi</i>	135
55	4.49	Tabular representation of HPLC chromatogram	138

## LIST OF FIGURES

S.No.	Figure No.	Title	Page No.
1	3.1	Routine colony maintenance of mosquito vectors <i>Ae. aegypti</i> , <i>Cx. quinquefasciatus</i> and <i>An. stephensi</i>	33
2	3.2	Representation of selected mosquito vectors	33
3	3.3	Representation of best selected plants for larvicidal activity	34
4	3.4	Experimental set up of larvicidal testing	36
5	3.5	Demonstration of <i>Channa punctuates</i> fish for Histopathological testing	38
6	4.1	Median lethal concentrations (LC <sub>50</sub> and LC <sub>90</sub> ) of different solvent extract of <i>Calotropis procera</i>	100
7	4.2	Median lethal concentrations (LC <sub>50</sub> and LC <sub>90</sub> ) of different solvent extracts of <i>Calotropis procera</i> against filaria vector, <i>Cx. quinquefasciatus</i>	104
8	4.3	Median lethal concentration (LC <sub>50</sub> and LC <sub>90</sub> ) of different solvent extracts of <i>Calotropis procera</i> leaves against malaria vector, <i>An. stephensi</i>	108
9	4.4	The 24h mortality of mosquito vectors, <i>Ae. aegypti</i> , <i>Cx. quinquefasciatus</i> and <i>An. stephensi</i> in various concentrations of different solvent extracts of <i>Calotropis procera</i> leaves	110
10	4.5	Median Lethal concentration (LC <sub>50</sub> and LC <sub>90</sub> ) of <i>Aegle marmelos</i> leaves against dengue vector, <i>Ae. aegypti</i> .	112

11	4.6	Median lethal concentration of different solvents of <i>Aegle marmelos</i> leaves against filaria vector, <i>Cx. quinquefasciatus</i> .	116
12	4.7	Median lethal concentration (LC <sub>50</sub> and LC <sub>90</sub> ) of different solvents of <i>Aegle marmelos</i> leaves against malaria vector, <i>An. stephensi</i> .	120
13	4.8	The 24h mortality of mosquito vectors, <i>Ae. aegypti</i> , <i>Cx. quinquefasciatus</i> and <i>An. stephensi</i> in various concentration of different solvent extracts of <i>Aegle marmelos</i> leaves	123
14	4.9	Median Lethal concentration of different solvents of <i>Agave americana</i> leaves against dengue vector, <i>Ae. aegypti</i> .	125
15	4.10	Median Lethal concentration (LC <sub>50</sub> and LC <sub>90</sub> ) different solvents of <i>Agave americana</i> leaves against filaria vector, <i>Cx. quinquefasciatus</i>	129
16	4.11	Median Lethal concentration (LC <sub>50</sub> and LC <sub>90</sub> ) of different solvents extract of <i>Agave americana</i> leaves against malaria vector, <i>An. stephensi</i> .	133
17	4.12	The 24h mortality of mosquito vectors, <i>Ae. aegypti</i> , <i>Cx. quinquefasciatus</i> and <i>An. stephensi</i> in various concentration of different solvent extracts of <i>Agave americana</i> leaves	133
18	4.13	HPLC chromatogram	136
19	4.14	Standard graph of mermelosin with regression coefficient R <sup>2</sup> = 0.9932	136
20	4.15	Chromatogram of obtained peak of ethanol extract of <i>Aegle marmelos</i> plant	137
21	4.16	Comparative histology of ethanol extract of <i>Aegle marmelos</i> in the liver of fish ( <i>Channa punctuates</i> )	140



# CHAPTER 1

## INTRODUCTION

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### 1.1 Mosquito-borne diseases

So far, there are approximately 3,601 recognized species of mosquito spread worldwide, out of which about 404 species are found in India, representing the diverse and medically important fly family Culicidae (Wilkerson *et al.*, 2015). The family Culicidae is a monophyletic family categorized on the basis of molecular and genetic information. There are two divergent subfamilies named as Anophelinae and Culicinae (Pawlowski *et al.*, 1996, Miller *et al.*, 1997). The Culicidae constitutes a large group belonging to primitive lower Diptera and includes mosquitoes, biting and non-biting midges. Many adult female mosquitoes considered to be a public enemy are either vectors or carriers for the disease. These mosquitoes are also transmitting pathogens among natural reservoir animals and birds. Millions of people come in contact with vector borne illnesses every year and causing approximately one million deaths worldwide every year in developing countries including India. It is worth mentioning here that among the cases of malaria that come in the world, the number of children is very high and its effects on children are also terrible. Out of more than 3,600 known species of mosquito, three species (*Anopheles*, *Culex* and *Aedes*) are dominantly spreading diseases in humans. *Anopheles* mosquito vectors are the world's only known malaria mosquitoes with more than 450 species world wide. *Culex* species of mosquitoes transmit filariasis, West Nile virus and encephalitis with more than 750 species worldwide. *Aedes* mosquitoes, also called Asian tiger mosquito, carry zika virus infection, dengue and yellow fever virus. There are more than 950 known *Aedes* species worldwide.

Mosquitoes hunt down humans by implying variety of ways, including our body heat and the carbon dioxide in our breath. The necessary mouthparts for blood feeding are present in female mosquitoes only. The two tubes of the proboscis of mosquito, pierce into the skin for two purposes: one is to inject an enzyme and another for blood sucking. The blood is used as a protein source for the development of eggs rather than their own nourishment. Male and female both, feed on plant sugars and nectar. The mode of disease transmission



through mosquitoes is diverse. Malaria parasites enter a host while female mosquito feeds since the parasites are attached to the gut of mosquitoes and this is how the transmission takes place, in the case of malaria. However, in case of dengue and yellow fever, the entry of virus takes place when the mosquito feeds on an infected human and further it is transmitted through the mosquito's saliva to a subsequent target.

Mosquito-borne infectious diseases (MBIDs) often carry the world's largest health burden in terms of mortality and disability-adjusted life years, making prevention and control critical. Each year, about seven hundred million people encounter mosquito-borne illness, killing more than one million people in over 150 countries around the world. In 2019, there were an estimated 229 million new cases of malaria worldwide, resulting in 409,000 deaths. In 2015-2016 approximately one million people was infected due to the Zika virus epidemic and caused thousands of babies with birth defects. Dengue fever is prevalent in more than hundred countries and affects about half of the world's population. WHO reported the increase in number of dengue cases eight fold over the past two decades, from 505,430 cases in 2000, 2.4 million cases in 2010, and 5.2 million cases in 2019. The number of reported deaths increased from 960 to 4032 in the year 2000 and 2015. Approximately 40 million people in India suffer from mosquito borne diseases every year. All these diseases are transmitted by different mosquito species (Nivedita, 2012).

## **1.2 Vector Control**

Controlling mosquito vector is a global problem and hence there is a need of integrated approach for vector management to control vector-borne diseases and reducing the burden of mosquito-borne pathogens worldwide in recent decades. This vector control approach requires detailed knowledge of which mosquito populations are transmitting a given pathogen in a geographic region, as well as effective tools that consider the biology and ecology of vector species in their implementation. Accurate and updated information on local factors is an important part of the vector control approach.

It is well known that disease can be eradicated by eliminating the causative organism by vector mosquitoes. The vector control is limited by a number of problems resulting from altered insecticide resistance, behaviour of vector and drug resistance. Spraying insecticide residue method is commonly used to control vector but its usefulness is limited in controlling vector borne diseases. This is important because the vector does not exist indoors only and so it can not be controlled by indoor spraying alone.

### 1.3 Disadvantages of Chemical Control

Chemical insecticides are being used extensively over the past decades to control the major genera of mosquitoes namely *Anopheles*, *Aedes*, and *Culex*. The most commonly and widely used insecticides are carbamates, organophosphates, organochlorines and synthetic pyrethroids. Aforementioned insecticides are in use for controlling different mosquito species involved in the spread of vector borne illnesses such as dengue, lymphatic filariasis, malaria, Japanese encephalitis etc. (Cui *et al.*, 2006).

Based on studies conducted in recent past, the continuous use of chemical insecticides, develop resistance in major disease transmitting species of mosquitoes at various stages of their lifecycle. Toxic chemicals are disturbing the food chain and causing pollution to the environment. When spraying insecticide is used to control mosquito larvae in well, ponds and other bodies of water can create problems for human health, larvivorous fishes and other water animals. The excessive use of mosquito repellants is causing sickness in human population which needs treatment as observed in some of the cases. Moreover, the effect of mosquito repellents in children is more severe (Sharma, 2001). It has been observed from the literature that the xenobiotics induce genetic variations among the mosquito populations and being responsible for developing different mechanisms for resistance which includes the exposure of mosquito larvae to fluoranthene and copper, increasing resistance towards permethrin which is the most widely used insecticide against mosquitoes (Poupardin *et al.*, 2008). Continuous use of chemicals against mosquitoes is showing adverse effects with the following disadvantages:

- This method is very expensive to apply due to high cost of insecticide
- This method is polluting our environment
- Repeated exposure to insecticides builds up insecticide resistance in insects
- It kills other beneficial flora and fauna and Non-Target Organisms
- Insecticide sprays are poisonous to human beings

These issues highlight the need to develop new strategies for mosquito control.

### 1.4 Importance of Phytochemicals

The use of toxins from plant extracts for mosquito control has long been utilized. Herbal insecticides are mostly specific to the pests, non-toxic, easily available, cost effective, ecofriendly and harmless to non-target organisms. They are eco friendly and biodegradable. Therefore, the use of readily degradable plant matter is considered safest and most effective

methods of controlling pests and mosquito vectors (Sivagnaname & Kalyanasundaram, 2004; Su & Mulla, 1998; Piplani *et al.*, 2019).

Plant products play an alternative role to chemical insecticides in integrated vector management programs and can be used as insecticides to kill larvae as well as adult. On the basis of phytochemicals polarity, it can be extracted from the specific part or whole plant with different solvents as aqueous, ethanol, methanol, hexane etc. Phytochemicals acts like anti-mosquito toxins, growth inhibitors, repellent, chemo sterilant and attractant. The herbal products interferes in the mechanism of growth, reproduction and development of the mosquito vectors.

**Action mechanism of secondary plant metabolites:** At the molecular level, it is still so poorly understood about the action mechanism of the secondary metabolites of plants against mosquito larvae. Most of the secondary plant metabolites have interference in the central nervous system (CNS) *via* respiratory absorption or skin, intoxication, inhibition of acetylcholinesterase (AChE) like some insecticides as carbamate and organophosphates (Moyes *et al.*, 2017). On the other hand, some contact mechanisms of action also observed with predators create a disturbance on the GABA system, which moves towards seizures and death, preventing oviposition as involved in the disturbance in mitochondrial process and digestive system (Menezes, 2015).

Given the importance of larvicide from the botanical origin, the present investigation was therefore conducted to test the selected plant leaves by evaluating the effects of secondary metabolites for their larvicidal properties against immature stages of *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*.

**The objectives of the present study are consolidated as:**

1. In silico, screening of 15 commercially available plants for mosquito larvicidal properties against the larvae of *Aedes*, *Culex*, and *Anopheles* vector species of mosquitoes.
2. The efficacy of aqueous and ethanolic extracts of three best-selected plants (based on the result of in silico study), followed by Sub fractionation of three best extracts with polar and non polar solvents against the larvae of *Aedes*, *Culex* and *Anopheles* vector species of mosquitoes.
3. Formulation of the best fraction with proposed mechanistic action as a contact poison for *Aedes*, *Culex*, and *Anopheles* mosquito vectors.



## CHAPTER 2

# REVIEW OF LITERATURE

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As the germ theory of disease emerged, it was rapidly realised that mosquitoes can communicate fatal diseases on earth. Parasite was identified in malaria patient first time by Alphonse Leveran in 1880 and the distribution of malaria disease in birds was demonstrated by Ross in 1898. Soon after, in 1900, the US army doctor Walter Reed and his commission, expanding on Carlos Finley's research, showed that mosquitoes of the *Aedes* genus spread yellow fever (Reed *et al.*, 1900). Ross and Leveran each won the Nobel Prize for their contributions to science in 1902 and 1907, respectively. The related to study literature have presented and discussed under following headings:

### 2.1 Vector-Borne Diseases

### 2.2 Mosquito Borne Diseases

#### 2.1.1 Malaria

#### 2.1.2 Filariasis

#### 2.1.3 Dengue

#### 2.1.3 Japanese encephalitis

### 2.3 Vector Control Methods

### 2.4 Plant Extracts as Mosquito Larvicides

#### 2.4.1. Insecticidal properties of *Calotropis procera*

#### 2.4.2 Mosquitocidal activity of *Calotropis procera*

#### 2.4.3 Insecticidal activity of *Calotropis procera* against other insects

#### 2.4.4 Insecticidal properties of *Agave american*

#### 2.4.5 Mosquitocidal activity of *Agave americana*

#### 2.4.6 Insecticidal properties of *Agave americana* against other insects

#### 2.4.7 Insecticidal properties of *Aegle marmelos*

#### 2.4.8 Mosquitocidal activity of *Aegle marmelos*

#### 2.4.9 Insecticidal properties of *Aegle marmelos* against other insect

## 2.1 Vector Borne Diseases

Vectors are organisms capable of spreading infectious diseases from people to animals, that is known vector-borne diseases. These illnesses accounts approximately seventeen percent globally and impacts on the medical health as well as economy, particularly in underdeveloped countries where young children are disproportionately afflicted (WHO 2014, 2015). Because of their effect on vector survival and reproduction, biting and feeding habits, pathogen incubation and replication, and the efficiency of disease transmission among many hosts, vector-borne pathogens are especially susceptible to climatic conditions. Mosquitoes are the most well-known and researched vectors, transmitting both parasite and virus. India has the second-largest population in the entire world. The majority of vector-borne illnesses thrive in a number of geo-ecological situations. India has a wide variety of illnesses that are spread *via* mosquito-borne vectors. Important vector-borne disease in India includes malaria, dengue, chikungunya, Japanese encephalitis, kala azar, lymphatic filariasis. Cases and death due to vector borne disease in last few years are given in Table (2.1).

**Table 2.1 The status of cases due to vector borne diseases in India (<https://nvbdcp.gov.in>)**

Year	Malaria	Dengue	Chikungunya	Japanese encephalitis	Kala azar
2015	1169261	99913	3342	1730	8500
2016	1087285	129166	26364	1676	6249
2017	841665	188401	12548	2181	5758
2018	429528	101192	9756	1678	4386
2019	338494	157315	12205	2545	3145
2020	186532	44585	6324	729	1967
2021	161753	193245	11890	787	1276
2022	176522	233251	8067	1109	810

## 2.2 Mosquito Borne Diseases

Mosquitoes are small insects that are the largest vector species in the taxa of biological kingdom. According to latest report, 3601 species of mosquitoes have been recorded worldwide, divided into two sub-families of 11 Tribes and Genera. In India, there are almost four hundred four species, fifty Genera and sixty three species of *Anopheles* mosquito have been listed earlier. In insects, mosquito vector shows more diversity in comparison of other animals and connects with mammals as well as plants. Mosquitoes belong to Diptera order and Culicidae family. According to Borkent and Grimaldi (2004) and Poinar *et. al.*, (2000) Mesozoic era is known as the origin of mosquitoes.

Globally, mosquito borne illness is a major issue of health concern. The World Health Organization has called mosquitoes as the "Public enemy No.1", due to transmission of various deadly diseases (WHO 1996). Mosquito vectors are the root cause of many important diseases. The transmission pattern of vector borne diseases depends on the environmental climate and the population of the country.

**2.2.1 Malaria:** It is a major public health problem in different regions of the country. About 95% of the country's population lives in malaria endemic regions and 80% of the country's reported malaria is confined to regions where 20% of the population lives in tribal, hilly, difficult and inaccessible areas. Plasmodium genus generates the malaria through obligate intraerythrocytic protozoa. Humans can be infected by the following four species: *P. malariae*, *P. falciparum*, *P. vivax*, and *P. ovale*. Geographically, the distribution pattern of these four malaria parasites is different from each other and so these species are located in different areas. Malaria is commonly transmitted by *P. falciparum* and the female *Anopheles* mosquito vector is responsible for malaria diseases in human. Among the four hundred *Anopheles* species, sixty vectors are known important worldwide.

**2.2.2 Filariasis:** After malaria, this is the second worldwide spread mosquito borne disease. Filariasis is transmitted by the filariae and affects the humans as well as animals. Out of the 100 parasites (filarial), eight filarial species are mainly transmit the infection in people. According to World Health Organization, Lymphatic filariasis (LF) is the major responsible disease for the disability by which approximately forty million persons affected worldwide. Mainly, *Wuchereria bancrofti*, *Brugia timori* and *Brugia malayi* are three parasites which cause the lymphatic filariasis in the world. *W. bancrofti* is the commonly found species (98 %) in India, while *B. malayi* (2 percent) is found in some areas of Gujarat, Goa and Kerala. *Cx.*

*quinquefasciatus* is the primary mosquito vector of *W. bancrofti* as well as *Mansonia annulifera* of *B. malayi*. These nematodes are thread-like worms which are found in the lymphatic system and lead to the *elephantiasis* syndrome. Lymphatic filariasis (LF) is spread due to the presence of larvae on the skin of worm which are deposit after the biting process of an infected female mosquito. The worm larva then passes towards the lymphatic system until it becomes changes into an adult stage which is four to ten centi meter long. Million worm larvae are produced by the adults which is known microfilariae and migrates towards the lymphatic system and blood stream. This infection is transmitted through the *Culex quinquefasciatus*, *Mansonia annulifera* and *M. uniformis* from one infected people to another people

The WHO envisioned that Lymphatic filariasis is located almost eighty sub-tropical and tropical international locations with one hundred twenty million inflamed instances, and with a billion human being are found at risk conditions, in which nine hundred fourty seven million human beings are found in danger zone, while fourty million human beings are injured *via* way of means of this contamination. Four locations India, Indonesia, Bangladesh and Nigeria make contributions approximately 70% of the LF contamination withinside the world. In India, indigenous instances were reported approximately two hundread fifty seven districts in twenty one States/Union Territories namely Delhi, Haryana, Punjab, Jammu & Kashmir, Uttaranchal, Chandigarh, Himachal Pradesh, Rajasthan and North-Eastern States are recognized to be loose from endemic filaria contamination. The cases of filarial infections were listed from some states in India as Jharkhand, Assam, Bihar, Maharashtra, Chhattisgarh, Goa, Uttar Pradesh, Gujarat, Kerala, Madhya Pradesh, Karnataka, Andhra Pradesh, Tamil Nadu, Orissa, West Bengal, Andaman & Nicobar Islands, Pondicherry, Dadra & Nagar Haveli, Lakshadweep and Daman & Diu (NVBDCP, 2022).

**2.2.3 Dengue:** It is an acute febrile mosquito-borne disease caused by four serotypes (DENV 1, 2, 3, and 4), and it belongs to the flaviviridae family and the genus flavivirus (Westaway *et al.*, 1985). All serotypes originated from sylvatic strains in the South-East Asia forests (Wang *et al.*, 2000). Every year approximately 50 to 100 million people suffers from newly infections, nearly 2.5 billion people are at risk and 20,000 die. India reported 193, 245 dengue cases during 2021 as against 44,585 cases in 2020. In particular, dengue viral infections are widely transmitted in tropical and subtropical environments and are transmitted by *Aedes aegypti*, which are mainly found in urban and semi-urban areas. The transmission of

dengue virus in human comes through the *Ae aegypti* mosquito (Linnaeus, 1762). Dengue fever is the world's fastest spreading vector-borne viral disease globally.

**2.2.4 Japanese encephalitis:** JE is a zoonotic viral infection, which is spread by the *Culex vishnui* subgroup mosquito vector species and Japanese encephalitis virus (JEV) is responsible for the disease. This virus (JEV) is a single stranded RNA. Japanese encephalitis disease affects the animals as well as humans. WHO estimates approximately sixty thousand JE cases in the world, annually. The threatened people are belongs to stay and work in rural areas, like pig farms and rice fields. The first JE case was reported in 1955 from Tamil Nadu. Then in 1973 there were reports from Bankura and West Bengal's Burdwan (Kumari R and Joshi P, 2012). Presently, JE disease is nationwide, mainly in the Eastern areas. In India, mainly found *Cx. tritaeniorhynchus*, *Cx. vishnui* and *Cx. pseudovishnui* three species of *Culex*, among them *Cx. vishnui* subgroup have been listed as main mosquito vector of Japanese encephalitis. *Cx. gelidus* and *Cx. epidesmus* species were involved in the transmission of JE.

### **2.3 Vector Control Methods**

The control of vector has been around the twenty century. Dichloro diphenyl trichloro ethane (DDT) used to reduce the mosquito vectors relied primarily of breeding sites by environmental management. Presently, herbal insecticides are used in various countries such as d-limonene camphor, Nicotine, Quassia, Turpentine, Pyrethrum, Azadirachtin, Chrysanthemum, and Derris (Rahuman *et. al.*, 2008). Ancient time (1950), Dichloro diphenyl trichloro ethane (DDT) and some other chemical or synthetic organochloride were considerably used to create the disturbance in transmission of mosquito borne diseases by reducing density and contact of human with the vector. In 1970, the burden of vector borne diseases increased suddenly due to resistance occurs against the insecticides, so the change in strategies were became necessary to control the mosquito vector (WHO 2005). One of the major drawbacks with the earlier organochlorine chemical insecticides is that they remain surround the environment for a considerable period and interact with various components of the ecosphere, thus resulting in an adverse effect on the ecosystem. Continued use of organochlorine pesticides damages liver, kidney, nervous system, thyroid, bladder and also causing cancer in human. Similarly, these insecticides causing liver and kidney cancer in animals. Due to its effects on non-targeting animals, the entire ecosystem is getting disturbed. Many vectors and pest species have developed physiological resistance to these compounds (Brown, 1986). To avoid the side effects caused by insecticides, scientists started to think



about the use of alternative methods to control the mosquito larvae as part of Integrated Programme (Ghosh *et. al.*, 2012).

## 2.4 Plant Extracts as Mosquito Larvicides

To see the larvicidal activities of plants, humans have used different plant parts, extracts, products and secondary metabolites to control the insects since ancient times. One report is credited on the basis of herbal product against the larvae of mosquito (Campbell *et al.*, 1933) which concluded about the plant alkaloids that the methyl anabasine, nicotine and lupine shows effective results against the of *Cx. pipiens* and *Cx. quinquefasciatus* larvae. Based on the objectives of the present research work (mentioned in chapter 1), the study has been done to merge the literature source available on the efficacy of eco-friendly *Calotropis procera* (Safed aak), *Agave Americana* (Century plant) and *Aegle marmelos* (Bael) for controlling mosquitoes and another important insect pests in chronological order (Table 2.2).

**Table 2.2 Taxonomic hierarchy of selected plants**

Sub Kingdom	Division	Order	Family	Genus	Species	Reference	
Viridi Plantae	Magnolio phyta	<b><i>Calotropis procera</i> (Safed aak)</b>					
		Sapindales	Apocynaceae	<i>Calotropis</i>	<i>procera</i>	<a href="https://en.wikipedia.org">https://en.wikipedia.org</a>	
		<b><i>Aegle marmelos</i> (Bael)</b>					
		Asparagales	Rutaceae	<i>Aegle</i>	<i>marmelos</i>	Kausik <i>et al.</i> , 2019	
		<b><i>Agave americana</i> (Century plant)</b>					
		Gentianales	Asparagaceae	<i>Agave</i>	<i>americana</i>	<a href="https://en.wikipedia.org">https://en.wikipedia.org</a>	

### 2.4.1 Insecticidal properties of *Calotropis procera* (Safed aak)

Calotrope and small crown flower is the common name of *Calotropis procera*. The plant is nearby to West Africa and having a long distance to south, North and southern Asia, East Africa, Indochina to Malaysia, Arabian Peninsula and Pakistan (Goyal and Mathur, 2011).

This plant grows mostly in drained soils area where as the two thousand (2000) mm precipitation occurs annually. *C. procera* is a 2 to 4 meter tall shrub. The colour of leaves was waxy green and the bark was light gray in colour. After the cutting of the plant parts there was white milk juice was also flow which is known as giant milkweed. The various plant parts are considered poisonous. From this species, various chemical compounds such as terpenoides, flavonoids, phenolic compounds, and cardiac glycosides have been isolated (Mueen *et al.*, 2005).The sap occurs calotropin compound, which creates the threaten issues for the heart by increases the non comfortability in human being. Literature study suggests that the parts and milkweed of *C. procera* has larvicidal activities.

#### **2.4.2 Mosquitocidal activity of *Calotropis procera***

This study focuses on reviewing the larvicidal properties of *C. procera*. The role of *C. procera* larvicide reported earlier (approx. 25 years ago) to control the mosquito vectors (Girdhar *et al.*, 1984). The milky sap from the plant, that represents the plant's own defense against insects (Larhsini *et al.*, 1997; Ramos *et al.*, 2006; Konno, 2011). Kumar *et al.*, (2022) also compiled a summary of research conducted over the past two decades on larval, insecticidal and adulticidal characteristics.

Markouk *et al.*, (2000) studied on the sixteen plant extracts from four medicinal potential plants: *C. procera*, *S. elaeagnifolium*, *Cotula cinerea* and *Solanum sodomaeum* against larvae of *An. labranchiae*. Nine extracts showed high larvicidal activity with LC<sub>50</sub> (24hrs) ranging from 28-325 ppm. The *C. procera* showed higher LC<sub>50</sub> value with aqueous latex against *A. labranchiae*, while the ethanolic extract of root showed lower LC<sub>50</sub> against *Anopheles* mosquito.

Singhi *et al.*, (2004) observed that when water was mixed with the aqueous latex extract, against the oviposition of pregnant *Ae. aegypti* female mosquito, showed that egg laying females could discriminate between extract concentrations and lay eggs on media with the lowest larvicidal concentrations.

The results of Singh *et al.*, (2005) studied that the methanol extract possesses more potent larvicidal activity than crude extract in *C. procera* plant. Cent percent mortality was observed in the early larval stage after 72 hours of exposure period in *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* with 3% concentration of crude leaf extracts. Methanolic extract at the concentration of 0.25% also produced 100% mortality of all stages of *An. stephensi*. In the case of *Aedes* and *Culex* the mortality of the third and fourth instar remained less than

100%. The LC<sub>90</sub> was 0.23% with methanolic extract while crude extracts required more than 3% concentration for causing 100% mortality of mosquito larvae.

Ramos *et al.*, (2006) recorded that the *C. procera* affects larvae development and suppresses egg hatching and 100 percent mortality in third stage larvae of *Ae. aegypti* after 5 min.

Singhi *et al.*, (2006) mentioned that *C. procera* latex states that it has larvicidal activity against all three major vectors: *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* in India. Fourteen various solvents were used to dissolve the latex part at 1000 ppm and concluded the 100% mortality in methanolic extract against *Ae. aegypti* larvae. The results explained that *An. stephensi* are lesser susceptible than *Cx. quinquefasciatus* at same concentrations of latex.

Marcio *et al.*, (2006) observed that the latex part of the *C. procera* caused cent percent larval death in 3<sup>rd</sup> instar *Ae. aegypti* larvae in a short span of time, and mostly individuals grown under laboratory conditions either died before second instar stage or remained at the first instar.

Elimam *et al.*, (2009) evaluated the larvicidal activity of *C. procera* against second, third, and fourth instar larvae of *An. arabiensis* and *Cx. quinquefasciatus* after 24 hours of treatment. At various concentrations, the larvicidal activity of third instar larvae of each mosquito species against *An. arabiensis* and *Cx. quinquefasciatus* was extracted. The calculated LC<sub>50</sub>–LC<sub>90</sub> values were found to be increased order for 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instar larvae respectively, of *An. arabiensis* and *Cx. quinquefasciatus*. 50% of adult emergence inhibition was also shown at different concentration against *An. arabiensis* and *Cx. quinquefasciatus*, respectively.

Shahi *et al.*, (2010) studied the larvicidal activity with the latex and the methanol extracts of *C. procera* leaves against *An. stephensi* and *Cx. quinquefasciatus*. Methanolic extract of latex were found more effective against *An. stephensi* than *Cx. quinquefasciatus* as a larvicide.

Oladimeji *et al.*, (2012) investigated the mosquitocidal potential against early 4<sup>th</sup> instar larvae of *An. gambiae* mosquito with crude extracts of 10 Nigerian plants. At five percent only *Carica papaya* and *Dacryodes edulis* showed significant larvicidal activity. However, at ten percent w/v, among the 10 plants only seven found highly effective after 24hrs incubation time.

Kumar *et al.*, (2012) using an in vitro method in *C. procera* leaves and described the phytoconstitutes and potential to control the mosquito. An aqueous extract of this plant (1,000 ppm) showed cent percent larvicidal activity against the 4<sup>th</sup> instar larvae of *Cx. gelidus* and *Cx. tritaeniorhynchus*. The extract of egg treatment of mosquito eggs showed cent percent ovicidal activity.

The larvicidal efficacy of two solvent extracts (aqueous and organic) from the seeds, leaves, and flowers of three desert medicinal potential plants, including *C. procera*, *Prosopis juliflora*, and *Tephrosia purpurea*, was studied by Bansal *et al.*, (2012). Different solvent extracts of *C. procera* seeds were tested, and after careful analysis, it was determined that the methanol and acetone extracts were the most effective against all mosquito species. In terms of mosquito control, petroleum ether extract fared better than aqueous extracts.

Tahir *et al.*, (2013) concluded the toxic effects of *C. procera* upon the mortality of *Cx. quinquefasciatus* larvae. Three concentrations (0.5%, 0.25% and 0.1%) were tested against the larvae. The latex concluded higher larval mortality in comparison of rubber free latex. The result assessed the effectiveness of rubber free latex and the whole latex showed cent percent mortality within 24hrs.

Singhi *et al.*, (2015) explored the larvicidal activity of *C. procera* insecticidal activity against *Ae. aegypti* with respect to different responses. The hexane extract of plant leaves were used and screened their larvicidal property. The hexane extract of *C. procera* leaves exhibited the LC<sub>50</sub> and LC<sub>90</sub> values 78.39 and 100.60ppm, respectively. Prolonged exposure of larvae to the extract increased the toxic potential of the extract and decreased the LC<sub>50</sub> values by 2.3%.

Freitas *et al.*, (2016) reported the fifteen chitinase isoforms in *C. procera* latex.

Aqsa Butt *et al.*, (2016) assessed the larvicidal properties of *C. procera* leaves and stem against *Ae. aegypti*. Larvicidal effect of leaves and stem extract of this plant against *Ae. aegypti* showed the increase order of mortality rate with the concentration respectively.

Mishra, (2017) showed high level toxicity of aqueous extract of the *C. procera* leaves against the *Cx. quinquefasciatus* larvae. LC<sub>50</sub> values were shown the increase order with concentration for second, third and fourth stage of *Cx. quinquefasciatus* larvae. The LC<sub>90</sub> values were also increased for 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instar larvae of *Cx. quinquefasciatus*, respectively. The LC<sub>90</sub> values indicated that second instar larvae were more susceptible than the third instar and the later was more susceptible than the fourth instar.

Funmilayo *et al.*, (2020) screened the methanolic extracts of 15 plants for larvicidal effects against the fourth instar larvae of *Cx. quinquefasciatus*. *C. procera* leaves extract, *Thevetia neriifolia* and the *Solanum macrocarpon* were found to be the highly active. After 48 hrs, the methanol extracts of the plant extracts had 1 or 2 highly active fractions. The n-hexane fractions of *S. macrocarpon* and *Spondias mombin* were found to be the highly active.

Yakubu *et al.*, (2021) evaluated the larvicidal activity of *C. procera* plant against larvae of *Cx. quinquefasciatus* and *Ae. aegypti*. The leaves were collected and extracted by using

petroleum ether solvent. Probit analysis of the result shows that the LC<sub>50</sub> value of *C. procera* extract was highest against *Ae. aegypti* (0.116 mg/ml).

#### **2.4.3 Insecticidal activity of *Calotropis procera* against other insects**

Salunke *et al.*, (2005) observed the efficacy of flavonoids between the *C. procera* and *Callosobruchus chinensis*, which fed on *Vigna radiate* (mung beans). On the basis of dose and exposure time, *C. procera* flavonoids showed higher toxicity in comparison of other adults and eggs flavonoids of *Callosobruchus chinensis* for at 10mg/ml doses.

Begum *et al.*, (2010) screened the ethanolic leaves extract of *Annona squamosa* and *C. procera* for the insecticidal testing against house fly (*Musca domestica*). The 3<sup>rd</sup> instar house flies larvae were used for the larvicidal experiments with various doses of leaf extracts. The LC<sub>50</sub> values of the leaves extract of *C. procera* showed higher larvicidal activity than *A. squamosa*.

Nenaah, (2013) extracted the extract of methanolic leaves, latex protein fraction and *C. procera* flavonoids were used to assess the larvicidal efficacy of *Sitophilus oryzae* and *Rhyzopertha dominica*. At the concentration of 5 ml/cm<sup>2</sup> the percentage of mortality were found to be 86.0, 77.6 and 61.0 in Cf, Lp and methanol extract, against *S. oryzae* respectively. The susceptibility status of *R. dominica* was lower than *S. oryzae*, where percentage of mortality was ranged between 53.8–64.2. Dietary study results showed that the test product at sublethal concentrations showed correlation with the growth and feeding rate of insects. Ibrahim *et al.*, (2017) studied on the ethanolic extracts of *C. procera* and *Khaya senegalensis* leaves for their phytoconstitute and larvicidal activities against cowpea weevils (*Callosobruchus maculatus*). The extracts of both plants at different concentrations were also investigated for their insecticidal effect against *C. maculatus*. The significant effect was showed (P ≤0.05) on the mortality rate of *C. maculatus* on the insect pests. The findings indicated that the plant extracts were highly toxic against *C. maculatus* of cowpea. The average oviposition of *C. maculatus* was observed during the research work which indicated that the extract has a significant effect against oviposition in the storage period. After treatment, oviposition proportionally associated with time intervals, which concluded that the oviposition rate was found to be higher in T1 and T2 than T3 (sample codes) preferably. The average number of holes indicated the significant effect of plant extracts on weight reduction during the trial period in which the higher efficacy of the treatments ranged between 0.60 (0.00) to 0.08 (0.28) in T1 and 0.16 (0.38) to 0.16 (0.57) in T2 to 0.00 (0.57) in T3 which was considered less infested by *C. maculatus* when compared with control.

#### **2.4.4 Insecticidal properties of *Agave americana***

*Agave americana*, is also called as century plant, is belong to the Asparagaceae family a species of flowering plant. *A. americana* is familiar to US, Mexico, and Arizona. It has become assimilated in various regions like India, Australia, South America, West Indies, the parts of southern Mediterranean Basin, and Africa, China and Thailand. It is multi-annual, monocarpic, with large and succulent leaves, height reaches upto 2 meter. It is cultivated medicinally, for fodder, agriculturally, as an ornamental as well as to control the erosion. Humans as well as animals both can be injured due to the shapness of margins and spines in leaves. Soap is prepared by the root and leaf extracts. Globally, it is cultivated as a fodder plant. Although, its leaf juices having anti fungal, anti inflammatory and anti bacterial properties.

#### **2.4.5 Larvicidal activity of *Agave americana***

In experiments conducted by Dharmshaktu *et al.*, (1987), *A. americana* leaf extract was found to be 100% effective against 4<sup>th</sup> instar *Anopheles*, *Aedes*, and *Culex* larvae at a concentration of 0.08% in 24 and 48 hours. After 24 hrs, the cent percent mortality were found in seed extract with 1:200 proportion against *Anopheles* and *Aedes* and 56 percent for *Culex*, while room temperature were showed significantly role to expose the forth instar larvae. After ten hrs, *Anopheles* larvae were exposed with 100% mortality while *Aedes* larvae exposed after 17hrs with 1:200 dilutions with water.

Singh *et al.*, (2014) studied the *A.sisalana* leaves extract against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. Initial observations mentioned that the leaf extract produced 100% mortality (at 2% dilution) against third instar *An. stephensi* larvae and cent percent mortality in one percent dilution showed effectiveness against *Ae. aegypti* and *Cx. quinquefasciatus*. For the larvicidal bioassays, the lethal concentration value of leaf extracts of dried crude, petroleum ether and methanol were showed significantly difference in late third or fourth instar larvae of *Cx. quinquefasciatus*, *An. stephensi*, and *Ae. aegypti*. The present study revealed that *A. sisalana* leaf extract possesses the potencial larvicidal property against *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi*.

Nunes *et al.*, (2015) studied the insecticidal effect of *Agave sisalana* crude extracts against *Ae. aegypti*. 4<sup>th</sup> instar *Ae. aegypti* larvae were tested to various concentrations of crude extract of *A. sisalana* to determine the LC<sub>50</sub> values for 3, 6, 12, and 24 hours. In addition, histological changes were concluded by histopathological studies and Nitric Oxide produced by blood cells was measured after various exposure time to *A. sisalana* raw extract. Furthermore, flow

cytometry showed enlargement of cell in cellular necrosis in mosquito larvae, which exposed the crude extract of *A. sisalana*. The results indicate that crude extract of *A. sisalana* constitutes is an effective larvicide against *Ae. aegypti* mosquito due to its necrotic activity and production in blood cells.

Kajla *et al.*, (2016) found that aqueous leaf extracts of *A. angustifolia* had potential larvicidal activity against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* larvae within 12 hours. Larvicidal activity of plants is observed only in the summer season and is higher in the winter season which depends on the temperature. The larvicide component of *A. angustifolia* is induced by varying the ambient temperature and is stable at high temperatures. The larvicidal properties was induced when *A. angustifolia* were kept at 37°C whereas the controls were grown outdoors at lower temperature. The initial incubation of the plant extract at hundred degree temperature for 1 h resulted sixty percent mortality in 12 hr, and a gradual increase in mortality to 100% in 24hrs. Furthermore, the dry powder herbal formulation of this plant, showed potent larvicidal activity even after long storage periods. These results indicate that *A. angustifolia* plant is temperature inducible and its secondary metabolites can assist in the preparation to control the mosquito vector programs.

#### **2.4.6 Insecticidal properties of *Agave americana* against other insects**

Guleria and Kumar, (2009) studied the fungal preventing activity of *A. americana* leaf extract against *Alternaria brassicae*, the main causative agent of *Alternaria* blight of *Brassica juncea*. *A. americana* methanolic leaf extract have antifungal activity against *A. brassicae*. Among three fractions, only methanol fraction exhibited the strongest antifungal property by inhibiting *A. brassicae* growth. The methanol fraction of this plant was further fractionated into sub-fractions (I, II, III, VI) by using column liquid chromatography. Among these sub-fractions, II recorded a extreme inhibitory effect on *A. brassicae* germination. At a 40 µg/ml concentration, it inhibited the development of lesions caused by *Alternaria* blight disease.

Maazoun *et al.*, (2019) looked into the insecticide's effectiveness against adult *Sitophilus oryzae*. Total phenols were found to be 14.70 0.31 mg GAE/g FW, total flavonoids 5.15 0.18 mg RE/g FW, and total saponins 10.32 0.20 mg OAE/g FW after extraction from *A. americana* leaves. Based on HPLC-ESI/TOF-MS analysis, flavonoid glycosides (kaempferol, quercetin, and isorhamnetin derivates) were identified as the key effective phytochemicals. Insect lethal concentration (LC<sub>50</sub>) and repellent effectiveness (RC<sub>50</sub>) values were determined to be 10.55 g per insect for the topical application method, 8.99 g/cm<sup>2</sup> for the treated filter-paper method, and 0.055 g/cm<sup>2</sup> for the repellent bioassay.

#### 2.4.7 Insecticidal properties of *Aegle marmelos*

*Aegle marmelos* is a member of the Rutaceae family and goes by the names bael and bel. Naturally occurring populations can be found throughout the Himalayas, Bengal, Central, and Southern India. The tree and its leaves are commonly used in Hindu rituals, so it is often planted near religious buildings. Each of its limbs is tipped with a sharp, straight spine. The bark is pliable, a pale grey colour, and peels off in large, irregular flakes. The leaves are trifoliolate and alternate in shape and a vibrant green. The flowers are a creamy green colour with a mildly sweet scent; the fruits are grey and globose with a woody rind; and the seeds are numerous, oblong, and compressed. The *A. marmelos* tree is originally from India but has become widely naturalised and cultivated across the Asia-Pacific region. It can reach a height of 10-12 metres. This is a staple of Ayurvedic and other alternative medicine practises. Many different chemical components of *A. marmelos* have been isolated.

The tree *A. marmelos* has been used for thousands of years in the Indian subcontinent and indo-china as a traditional medicinal plant. Historic mention of bael fruit has been traced to vedic times (2000-800 BC). It has been considered a sacred plant by Hindus, and it is grown in Indian temple gardens. All parts of the tree are commonly used in the treatment of different diseases. *Aegle* constituents are helps in cardiovascular diseases (Kakiuchi *et al.*, 1991), and wound healing (Udupa *et al.*, 1994). *A. marmelos* leaves have hypoglycemic effects (Santhoshkumari and Devi, 1990; Sharma *et al.*, 1996). Essential oils extracted from *A. marmelos* leaves have antifungal properties (Renu *et al.*, 1986; Rana *et al.*, 1997).

*A. marmelos* possess various insecticidal properties. Leaf extracts have acaricidal, larvicidal and insecticidal properties (Narasimhan and Mariappan, 1988; Hazarika *et al.*, 2000). Essential oil of *A. marmelos* leaves were reported to show insecticidal properties against four grain insect pests including *Callasobruchus chinensis*, *Sitophilus oryzae*, *Rhyzopertha dominica*, and *Tribolium castaneum*. Essential oil from the *A. marmelos* leaves have insect repellent activity while used against *S. oryzae* and *T. castaneum* (Mishra and Tripathi, 2011). *A. marmelos* contains several active compounds like alkaloids, terpenoids, coumarins, phenylpropanoids, tannins, polysaccharides and flavonoids. Compound aeglein, marmelosin, d-limonene and ethyl-p-cumarate of the leaves have shown pesticide, larvicidal and insecticidal activities. The present investigations on previous work done on insecticidal activities of *A. marmelos* with special reference to *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*, collected to understand the current status of knowledge on topics related to our studies.



#### 2.4.8 Mosquitocidal activity of *Aegle marmelos*

*A. marmelos* is very old associated with human civilization. It has been used as a traditional medicinal plant in Indian subcontinent and China. This plant also holds a high position in India from a religious point of view. Several studies conducted earlier around the world provide scientific evidence for the *A. marmelos* effect on mosquitoes and other insect pests.

Plant extracts from *A. marmelos*, *Andrographis lineata*, *Andrographis paniculata*, *Cocculus hirsutus*, *Eclipta prostrata*, and *Tagetes erecta* were tested on *An. subpictus* and *Cx. tritaeniorhynchus* fourth-stage larvae by Elango *et al.*, (2009). All extracts showed moderate larvicidal efficacy after 24 hours of exposure at 1,000 ppm. Larval mortality was highest with ethyl acetate on *A. marmelos*, *E. prostrata*, hexane, methanol of *A. paniculata* and *C. hirsutus* against *An. subpictus* larvae LC<sub>50</sub> value was 167.0, 78.2, 67.2, 142.8 ppm and LC<sub>90</sub> value was 588.3, 360.7, 371.9, and 830.0 ppm) while against *Cx. tritaeniorhynchus* larvae LC<sub>50</sub> value was 99.0, 119.8, 88.5, 105.1 ppm and LC<sub>90</sub> value was 479.2, 564.8, 416.3, and 507.8 ppm.

The larvicidal potential and smoke repellency action of *A. marmelos* and *Toddalia asiatica* at different doses (100, 80, 60, 40, and 20ppm) against all stages of *Ae. aegypti* larvae and pupae were investigated by Vineetha and Murugan, (2009). The 50% lethal concentration (LC<sub>50</sub>) for *A. marmelos* and *T. asiatica* extracts against first-instar larvae was 50.960ppm and 47.893ppm, respectively; for fourth-instar larvae, it was 60.7ppm and 61.2ppm; for third instar larvae, it was 56.6ppm and 54.4ppm; and for second instar larvae, it was 52.9ppm and 50.9ppm. The LC<sub>50</sub> and LC<sub>90</sub> values for *A. marmelos* and *T. asiatica* were 56.6 and 112.9 ppm and 53.6 and 116.2 ppm, respectively. The smoke of *T. asiatica* was found to be more toxic to *Ae. aegypti* than that of *A. marmelos*.

Patil *et al.*, (2010) tested on the early 4<sup>th</sup> instar larval stage of *Ae. aegypti* and *An. stephensi* of crude dichloromethane, chloroform and methanol extract of six indigenous plants (leaves and roots), *A. marmelos*, *C. gigantea*, *Balanites aegyptica*, *Nyctanthes arbor-tristis*, *Murraya koenigii* and *Plumbago zeylanica*. The 24 hrs exposure time of larval mortality was observed. All extracts were found effective against larvae tested. Methanol extracts had the highest larval mortality against *Ae. aegypti* of *B. aegyptica* roots and *P. zeylanica* roots, with LC<sub>50</sub> values of 289.59 mg/l and 169.61 mg/l, respectively, while *An. stephensi* had LC<sub>50</sub> values of 102.29 mg/l and 222.34 mg/l. Plant methanol extracts were found to be more effective than other extracts tested in this study.

Elango *et al.*, (2010) investigated the repellency of *Cx. tritaeniorhynchus* against ethyl acetate, acetone, and methanol extracts of *A. marmelos*, *Andrographis lineata*, *Cocculus hirsutus*, *A. paniculata*, *Tagetes erecta*, and *Eclipta prostrate*. The maximum repellency

effects were observed at 500ppm in *A. marmelos* methanol extracts and *A. lineata*, *C. hirsutus*, and *E. prostrata* ethyl acetate extracts, with the mean complete protection time ranging from 120 to 150 minutes. The *A. lineata* extract with ethyl acetate demonstrated 100% repellency in 120 minutes, while acetone extracts of *A. marmelos* and *C. hirsutus* and methanol extract of *T. erecta* demonstrated full protection in 90 minutes.

Inhibition of adult insecticidal properties and adult emergence rate of methanol, hexane, acetone, chloroform and ethyl acetate leaf extracts of *A. marmelos*, *Cocculus hirsutus*, *Eclipta prostrate*, *Andrographis lineata*, *Andrographis paniculata*, and *Tagetes erecta* were tested against *An. subpictus*. These phyto extracts exhibited adult insecticidal properties and emergence inhibition (EI) response after exposure to 1,000ppm for 24 hrs. The highest insecticidal activity of adult *An. subpictus* was found in the *T. erecta* methanol extract, *A. lineate* ethyl acetate extract, *A. paniculata* chloroform extract and *C. hirsutus* acetone extract (LC<sub>50</sub> 89.83, 126.9, 95.8, 109.4ppm; LC<sub>90</sub> 607.8, 542.9, 720.8, and 459.0ppm) respectively. Emergence Inhibition effect was found in the *A. marmelos* leaf acetone extract (EI<sub>50</sub> 128.14, EI<sub>90</sub> 713.53), ethyl acetate extract of *A. lineate* (EI<sub>50</sub> 79.39, EI<sub>90</sub> 293.70), *C. hirsutus* (EI<sub>50</sub> 143.97, EI<sub>90</sub> 682.72) and *T. erecta* methanol extracts, (EI<sub>50</sub> 92.82, EI<sub>90</sub> 582.59 ppm) (Elango *et al.*, 2011).

Dass and Mariappan, (2014) tested the pupicidal activity and larvicidal properties of *A. marmelos*, *Vitex negundo* and *Coleus aromaticus* leaf extract against second, third and fourth instars larvae of *Cx. quinquefasciatus*. The LC<sub>50</sub> values of *V. negundo* for 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> instar larvae and pupa were recorded as 66.3ppm, 74.0ppm, 84.36ppm and 133.3ppm respectively. While LC<sub>50</sub> value calculated for *A. marmelos* for 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> stage larvae and the pupa was 91.5ppm, 105.1ppm, 151.4ppm and pupa 203.7ppm respectively. In the same way LC<sub>50</sub> value obtained for *C. aromaticus* was 137.7 ppm for the second stage, 175 ppm for the third stage, 188.36 ppm for fourth stage and 221.4ppm for pupal stage.

Reegan *et al.*, (2015) assessed the egg laying behaviour and other related parameters of methanol, ethyl acetate and hexane leaves extract of *A. marmelos*, *Limonia acidissima*, *Sphaeranthus indicus*, *S. amaranthoides* and *Chromolaena odorata* against *Ae. aegypti* and *Cx. quinquefasciatus*. The five plant extracts were screened in this experiment, 100% oviposition and significantly ovicidal activity were found in *L. acidissima* hexane extract against the vectors and could be used to control the vectors.

The effectiveness against *Cx. quinquefasciatus* and *Ae. aegypti* was evaluated by Sharma *et al.*, (2017). The fresh leaves of *A. marmelos* were distilled in water to remove the essential oil. Both species of mosquitoes were tested with various concentrations of essential oil across

their life cycles. Experiments with a variety of mosquito populations revealed strikingly different levels of activity among the species. Essential oil was found to be an effective larvicidal and adulticide agent against *Cx. quinquefasciatus*. After 72 hours of exposure, the LC<sub>50</sub> value was calculated to be 121.8 ppm for larvae and 121.5 ppm for adults, and the essential oil proved effective as an ovicidal and repellent agent against *Ae. aegypti*. The calculated LC<sub>50</sub> value was 278.8 ppm after 72 hours of exposure and 1 hour of safety.

Sowmyashree *et al.*, (2019) investigated two plant essential oils (EO) of natural products *viz.*, *A. marmelos* and *P. guajava* to test the mosquito larvicidal activity against *An. stephensi*. Five different concentrations of the EOs were used to test the larvicidal activity against the fourth stage of larvae. The experiment proved the correlation between the larval mortality and concentrations, larval mortality was directly related to the exposure time and dose of the EOs. The LC<sub>50</sub> and LC<sub>90</sub> value of *A. marmelos* (EO) at 24hrs was 54.9ppm, 85.1ppm and at 48hrs was 53.9ppm, 74.3ppm, respectively. And the same values for *P. guajava* were 40.2 ppm, 56.4ppm and 38.0ppm, 51.5ppm, respectively. The results concluded that *An. stephensi* larvae were highly susceptible to the *P. guajava* essential oils than *A. marmelos*.

Dass *et al.*, (2022) assessed the effectiveness of methanolic extracts of *A. marmelos* and *Coleus aromaticus* against *Ae. aegypti* larvae and pupa. They recorded LC<sub>50</sub> values after 24 hours for 2<sup>nd</sup>, 3<sup>rd</sup> 4<sup>th</sup> instars, and pupa for leaf extracts of *A. marmelos* as 124.27ppm, 145.07ppm, 178.87ppm, and 225.99ppm, respectively. Similarly the LC<sub>50</sub> value for *C. aromaticus* plant extract was 62.46 ppm, 81.94 ppm, 101.19 ppm, and 124.34 ppm. The LC<sub>90</sub> for *A. marmelos* were 222.74ppm, 283.43ppm, 354.02ppm, and 439.73ppm. While for *C. aromaticus*, 162.87 ppm, 202.83ppm, 213.63ppm 254.14ppm LC<sub>90</sub> was recorded.

A field test was done by Selvan *et al.*, (2021) to observe larval mortality at 24 hrs, 48 hrs and 78hrs time and larval and reduction in pupal percentage due to both plants extracts in the unused cement tank and mini water pool in Tiruchirappalli and Puthanampatti district. The field tested showed different activities against larval and pupal stages of *Culex* spp. Therefore, *P. Trifoliata* and *L. acidissima* plant derived flavonoid compounds may be used to develop commercial mosquito larvicide to replace traditional synthetic chemicals synthetic chemicals, especially in integrated vector control programe.

#### 2.4.9 Insecticidal properties of *Aegle marmelos* against other insect

Hiremath *et al.*, (1997) assessed the insecticidal activities of 84 samples from 49 Indigenous plants in 30 families with methanol extracts by topical application method against the *Nilaparvata lugens* (brown plant hopper). At 0.5µg/female dose, the following 11 extracts indicated significant insecticidal activity: *Nerium indicum* stems, *Adhatoda vasica* leaves, *Annona squamosa* seeds showed cent percent mortality and *Clerodendrum inerme* whole plants, *Pongamia pinnata* seeds, *Prosopis chinensis* stems, *Vitex negundo* leaves recorded more than 90 percent mortality while *Azadirachta indica* seeds and stems, *A. marmelos* leaves and *Madhuca indica* seed oil calculated more than 80 percent mortality.

Samarasekera *et al.*, (2004) extracted new senecioate ester compounds, skimmiaepin A and C from the stem bark ethyl acetate extracts of *A. marmelos*. Both compounds exhibit moderate insecticidal activity than natural pyrethrum extract against *M. domestica* and *Phaedon cochleariae*.

The essential oil from the leaves of *A. marmelos* was tested by Kumar *et al.*, (2008) to see if it could be used to prevent the spread of insect pests like *Callosobruchus chinensis* in stored gramme and *Tribolium castaneum*, *Rhyzopertha dominica*, and *Sitophilus oryzae* in stored wheat. Gram and wheat samples were fumigated with 500 g per ml of essential oil from *A. marmelos* to see if it would deter the introduction of specific test insects (ppm). Wheat samples were infested with all insects except *T. castaneum* because the oil used greatly increased the feeding capacity of insects, resulting in damaged grain and decreased weight. Essential oil (EO) significantly decreased adult emergence and oviposition of *C. chinensis* in cowpea seeds across a range of doses. For two years, the gramme crop was safe from *C. chinensis*, and the wheat crop was safe from *R. dominica* and *S. oryzae* thanks to the essential oil used. The GC-MS (Gas Chromatography-Mass Spectrometry) results for the extracted oil indicated that the main player was the component Limonene (88%). Data regression analysis on treated cowpea confirmed a dose-dependent reduction in *C. chinensis* oviposition and adult emergence. The results showed that *A. marmelos* oil successfully deterred insects from destroying stored grains.

Ramya and Jayakumararaj, (2009) studied the insecticidal activity of twenty five medicinal plants against *Helicoverpa armigera*. Larval mortality at 1000ppm concentration was considered for the test. The results explained the larval mortality rate in the following order: *C. roseus*, *A. zeylanica*, *A. fruticose*, *D. metal*, *S. nigrum*, *O. canum*, *P. coleoides*, *O. sanctum*, *P. daemia*, *A. amara*, *G. sylvestre*, *C. halicacabum*, *V. negundo*, *A. indicum*, *C. tora*

*A. indica*, *S. trilobatum*, *A. paniculata*, *A. marmelos*, *T. terrestris*, *A. aspera* *M. azedarach* *A. lineata*, *S. surattrense*, and *A. lanata*.

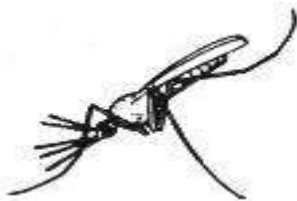
Arivoli and Tennyson, (2013) assessed the ovicidal activity of *S. litura* in 25 locally available plants with various extracts of dichloromethane, hexane, ethyl acetate and diethyl ether. They observed that the ethyl acetate extract of *A. marmelos*, diethyl ether extract of *Murraya koeingii* and hexane extract of *Cleistanthus collinus* exhibited the greatest ovicidal activity.

Mishra *et al.*, (2016) isolated the essential oil from *A. marmelos* leaves and observed insecticidal properties against *Tribolium castaneum*. The experiments showed that the *A. marmelos* essential oil possessed fumigation toxicity, spawning and stunting inhibition against *T. castaneum* pest. Mortality of pest during experiments increased with exposure time and dose used. After 48 hrs LC<sub>50</sub> value of essential oil was calculated 17.752 and 14.172FL against adults and larvae of *T. castaneum*, respectively. The essential oil reduced significantly in oviposition ( $F_{3,20} = 304.7$ ) of adults, pupation ( $F_{3,20} = 137.4$ ) and adult emergence ( $F_{3,20} = 225.6$ ) in larvae at the time of fumigation. The percent grains infection was reduced as 83.6 percent at 60 percent of the sub-lethal dose. Fumigation effect with sub-lethal dose of essential oil repressed AChE activity in insects. It was calculated as 81.48 and 54.32% in the control, after 24 hrs of fumigation.

Rejiniemon *et al.*, (2014) reported the larvicidal activity of *A. marmelos* leaves against *Helicoverpa armigera* and *Spodoptera litura* at 125, 250, 500 and 1000 ppm concentrations. The metabolite documented 63.6% and 71.8% larvicidal activities against *H. armigera* and *S. litura*, respectively at 1000 ppm. The LC<sub>50</sub> value was 786.16 and 696.37 ppm for *H. armigera* and *S. litura*, respectively.

The effects of *A. sativum* and *A. marmelos* on *Sitophilus zeamais* were studied by Chaubey (2017). Both plant's essential oils were effective in deterring adult *S. zeamais*. When tested in a fumigation toxicity assay against adults of *S. zeamais*, the LC<sub>50</sub> values for *A. marmelos* and *A. sativum* oils were 0.312 and 0.184 L cm<sup>3</sup> air, and 0.297 and 0.22 L cm<sup>3</sup> air, respectively. Adult *S. zeamais* were poisoned by the two essential oils due to their use as a fumigant and contact poison. When used as a contact poison, the LC<sub>50</sub> values for *A. sativum* and *A. marmelos* oils against adult *S. zeamais* were 0.208 and 0.116 L cm<sup>-2</sup> area after 24 hours and 0.227, 0.146, 6, 37 L cm<sup>-2</sup> area after 48 hours, respectively. When adults of *S. zeamais* were given a sub-lethal dose of either plant's essential oil, they stopped reproducing and stopped laying eggs. Toxic effects on the nervous system were reported after *A. sativum* and *A. marmelos* essential oils were used to fumigate *S. zeamais*. This toxicity was caused by an inhibition of acetylcholine esterase (AChE).

Snehlata and Sheel, (2020) investigated the larvicidal activity of *A. marmelos* acetone leaves extracts to control *Ostrinia nubilalis* and *Spodoptera littoralis*. The pest-infested stored food test samples were treated with fumigation of acetone extract of *A. marmelos* at 500 µg per ml. The treatment of this extract significantly affects the feeding behaviour of insect and prevents the grain damage and weight loss of food samples. Regression analysis also confirmed the reduction of oviposition and adult emergence of insect pests in doses dependent manner. After 72 hrs of incubation period at 10ppm the hatching percentage of *O. nubilalis* and *S. littoralis* was 79.75% and 76.35% respectively. The LC<sub>50</sub> value was therefore recorded at 72 hours of exposure time. While at 1000ppm dose of extract the hatching of *O. nubilalis* and *S. littoralis* was 34.85% and 33.35%. The larval hatching and survival rate decrease with increasing concentration of extract and with incubation period. The finding emphasizes the effectiveness of *A. marmelos* leaves as a treatment against pest infestation; it enhances the potential for use as an ecofriendly alternative to the chemicals to preserve stored food and grains.



## CHAPTER 3 MATERIALS AND METHODS

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Standard techniques were adopted to study the computational, entomological, toxicological and histopathological parameters. The present study was carried out with three plant species (*C. procera*, *A. americana* and *A. marmelos*) for testing the larvicidal potentials against three species of mosquitoes (*An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*).

### 3.1 Computational Study

#### 3.1.1 Ligands Retrieval and Preparation:

The ligand molecules were retrieved from Chemspider and the inhibitory protein molecules in 3D format were obtained from PubChem database.

#### 3.1.2 Molecular Docking of Target Proteins with Ligands

To analyze the binding mechanism of ligands and target proteins, molecular docking was performed. The activity of compounds was predicted using CB-Dock tool online server (<http://clab.labshare.cn/cb-dock/php/index.php>). The spectrum of the biological activity of the chemical compounds change with the respective compound which reflect the interactions of various compound with different biological existence. The intrinsic property of a compound is represented by its biological activity spectrum which depends on its structure and physio-chemical characteristics. CB-Dock is a method of docking of protein with ligand which identifies automatically, the binding sites and calculates the center and resize docking area to known ligand and hence completes the molecular docking process with open source program AutoDock Vina. According to the large scale benchmarks cavity focused docking increases the accurateness of blind docking. Hence, CB Dock tool facilitates blind docking method to predict the binding site of target proteins by the curvature-based cavity detection approach. Thus, CB-Dock tools are valuable to know the biological activity of chemical compounds. If

probability to be active ( $P > 0.7$ ) value is greater than 0.7, that means the molecule is showing activity.

### 3.1.3 Selection of the phytoconstituents of selected plants and target Proteins

Fifteen plants and their active compounds were selected based on a literature survey, which are having larvicidal properties against mosquito larvae. The active compounds of the plants and their target proteins are represented in Table 3.1

**Table 3.1: List of phytoconstituents of the selected plant leaves**

S. No.	Name of the plant	Common Name	Part used	Active compound	Reference
1	<i>Calotropis procera</i>	Safed aak	Leaves	ALP (Alkaline phosphatase), AP (acid phosphatase), ALT (alanine aminotransferase), AST (aspartate aminotransferase), total protein, total bilirubin, albumin; urs-19(29)-en-3-yl acetate, stigmasterol, b-sitosterol, urs-19(29)-en-3-b-ol and 3b,multiflorenol, 27-dihydroxy-urs-18-en-13,28-olide	Wadhvani <i>et al.</i> , 2021
2	<i>Ficus bengalensis</i>	Banyan	Leaves	20-tetratriacontene-2-one(1), 6-heptatriacontene-10-one (7), beta-sitosterol-alpha-D-glucose and meso-inositol pentatriacontan-5-one (13)	Yadav <i>et al.</i> , 2015
3	<i>Catharanthus roseus</i>	Sadabahar	Leaves	Amyrin acetate and oleanolic acid	Lahare <i>et al.</i> , 2020
4	<i>Datura stramonium</i>	Datura	Leaves	3-phenylacetoxy-6, 7-epoxynortropine and 7-hydroxyapoatropine, 3,7-dihydroxy-6 propionyloxytropine, 3-tigloyloxy- 6,7-epoxytropine,	Soni <i>et al.</i> , 2012



				6,7-dehydro-tigloyloxytropone, 3,7-dihydroxy-6-(2'-methylbutyryloxy), 3(3'methoxytropoyloxy) tropone, tropone,6,7dehydroapootropine, 3-tropoyloxy-6-isobutyryloxytropone, 3-tigloyloxy-6 isobutyryloxy-7-hydroxytropone, 3 $\beta$ -tropoyloxy-6 $\beta$ -isovaleroyloxytropone.	
5	<i>Mentha piperita</i>	Peppermint	Leaves	Piperitenone oxide, and 4-terpineol	Brahmi <i>et al.</i> , 2017
6	<i>Aloe vera</i>	Aloe	Leaves	Anthraquinones or phenoli compounds, lignin, tannic acids, polysaccharide, gly-89coproteins,saponins, sterols, amino acids and salicylic	Kahramanoglu <i>et al.</i> , 2019
7	<i>Eucalyptus camaldulensis</i>	Eucalyptus	Leaves	1,8cineole, $\gamma$ -Terpinene, $\alpha$ -Pinene and Globulol	Ghareeb <i>et al.</i> , 2018
8	<i>Aegle marmelos</i>	Bael	Leaves	5-isopropenyl-2-methyl-7-oxabicyclo (4.1.0) hepten-2-ol, $\beta$ -terpinyl acetate, 2,3-pinenediol,Rutin, $\beta$ -sitosterol, Skimmianine, Glycoside, Citronellal, Marmesinin, Eugenol, Marmelosin, Marmeline	Laphookhieo <i>et al.</i> , 2011
9	<i>Ocimum sanctum</i>	Tulsi	Leaves	Amines, imines (N – H str), Alkanes (-CH <sub>3</sub> ), alkenes (R <sub>1</sub> CH=CHR <sub>2</sub> ), Nitrates (O–NO <sub>2</sub> v), (O–H) oxygenated bonding, carboxylic acids, esters, ethers, C-N stretching alcohols	Baliga <i>et al.</i> , 2013
10	<i>Cannabis</i>	Bhang	Leaves	Trans-Anethol (19.83), Linalool	Elsohly <i>et al.</i> , 2014

	<i>sativa</i>			(57.11), $\gamma$ -terpinene (3.83), $\alpha$ -pinene (1.8), geranyl acetate(3.2)	
11	<i>Eichhornia crassipes</i>	Water hyacinth	Leaves	shikimic acid, $\beta$ -sitosterol, 2-hydroxy-8-(4-hydroxyphenyl)-1H-phenalen-1-one and 1, stigmasterol, 1-diphenyl-2-picrylhydrazyl	Jayanthi <i>et al.</i> , 2012
12	<i>Lantana camara</i>	Spanish flag	Leaves	Oleanonic acid, 22 $\beta$ -acetylantonic acid and 22 $\beta$ -dimethylacryloyloxylantonic acid, 1, 1-diphenyl-2-picrylhydrazyl (DPPH)	Es-Al <i>et al.</i> , 2019
13	<i>Achyranthes aspera</i>	Chaff-flower	Leaves	$\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)-( $\beta$ -D-glucopyranosyluronic acid)-(1 $\rightarrow$ 3)-oleanolic acid, and $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)-( $\beta$ -D-glucopyranosyluronic acid)-(1 $\rightarrow$ 3)-oleanolic acid-28-O- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranoside, 12-O-tetradecanoylphorbol-13-acetate, 10-tricosanone, 10-octacosanone and 4-tritriacontanone	Kamana <i>et al.</i> , 2014
14	<i>Allium ampeloprasum</i>	Elephant garlic	Bulb	Carbon tetrachloride, S-alk(en)yl-L-cysteine sulfoxides (ACSOs), polyphenols, Diallyl thiosulphinate (allicin), methyl allyl thiosulphinate, and allyl methyl thiosulphinate, D-limonene, Beta-pinene, Trans-caryophyllene, Dimethyl trisulfid, Caryophyllene oxide, Elemene, Dimethyl tetrasulphide, Alpha-pinene,	Abd <i>et al.</i> , 2013

				Gamma terpinene, Beta-myrcene, Farnesene and Alpha-terpineol	
15	<i>Agave americana</i>	Century plant	Leaves	Cantalasaponin-1, inulin,	Tinto <i>et al.</i> , 2005

### 3.1.4 Selection of Proteins:

Three best proteins were retrieved from PDB (Protein data base) on the basis of resolution. 6P2E, 6DU6 and 5V13 proteins were retrieved from *Ae. aegypti* mosquito. 5W1U, 2C9K and 2WLS were retrieved from *Cx. quinquefasciatus* and 4OKV, 3NGV and 3NHT were retrieved from *An. stephensi*. The detailed information of these proteins was described in the Table 3.2.

**Table 3.2 Details of the proteins retrieved from *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles stephensi***

Name of Mosquito Vector Species	Protein Name	PDB ID of Protein	Reference
<i>Ae. aegypti</i>	Transport protein	6P2E	<a href="https://www.rcsb.org">https://www.rcsb.org</a>
	Transferase	6DU6	
	Mosquito juvenile hormone-binding protein	5V13	
<i>Cx. quinquefasciatus</i>	Carboxyesterase B2	5W1U	
	Mosquito larvicidal toxin protein	2C9K	
	Acetylcholinesterase	2WLS	
<i>An. stephensi</i>	Anti-platelet protein	4OKV	
	Transport protein	3NGV	
	Transport protein	3NHT	

## **3.2 Entomological Study**

The entomological investigations were undertaken to achieve the above mentioned objectives. Mosquito larvae were reared and tested against different plant extracts according to WHO recommended guidelines (WHO, 2005). Separate insectary was maintained by taking some adults and larvae from the pre existing NCDC insectary so that sufficient quantity of larvae could be obtained for the experiment purpose.

### **3.2.1 *An. stephensi***

#### **2.1.1 Rearing and Maintenance of *An. stephensi* Mosquitoes**

The larval stages were reared in white round enamel bowls (30cm diameter and 10cm depth) having chlorine free water. Yeast as food was given to larvae everyday. Mosquito insectary was established at  $27\pm 2^{\circ}\text{C}$  temperature with 14:10 photoperiod. Humidity was kept between 75 to 85 percent. The water was changed on every day or every other day. Presence of eggs was checked regularly and died larvae were removed everyday. To avoid contamination, net cloth was used to cover all water bowls. Pupae were collected two times a day and transferred to white plastic bowl (10cm diameter) having 500 ml water. White plastic bowl with pupae was kept in 12×12×12 inches size mosquito cages for adult emergence. Inside adult mosquito cage, soaked cotton balls were kept to feed adult mosquitoes with sugar solution. These cotton balls were dipped in glucose (10%) solution, and then cotton ball was placed in cage. Cotton balls were changed on daily basis. On the second day of post emergence, adult females provided blood as a meal for egg laying. In every 48 hrs, during night a rabbit was kept in adults cage in the iron frames to feed blood to the naturally mated adult female mosquitoes. Adult mosquitoes were maintained at the same environmental conditions as larvae.

#### **2.1.2 Egg Production**

Blood fed females were kept in cage provided with a small water bowl for oviposition. Egg counts were made daily from each group over a 4-6 days span. Average numbers of eggs laid were observed after 24, 48 and 72 hours. The number of first instar larvae hatched after 72 hours.

### **2.1.3 Larvae**

First stage larvae that emerged after 48-72 hours were placed in an enamel bowl with water. The yeast powder was added in water bowl for feeding the larvae. The water from enamel bowl was changed every alternate day. There were fourth instar larvae with an average of 14-16 days before they converted into pupae. Late third instars larvae and early 4<sup>th</sup> instars larvae were selected for the larvicidal testing. Few larvae were kept for pupation to run the colony. Upon pupation, the pupae were placed in plastic bowls of water within an adult mosquito cloth cage to ensure the emergence of adults in cloth cage. The male adults who emerged were again provided with source of feeding 10 percent glucose solution and female were provided with blood feeding. A bowl of water kept in the cage for oviposition, to continue the life cycle in the insectary. Abiotic factors (temperature and relative humidity) were taken into account throughout the life cycle at constant temperature ( $27\pm 2^{\circ}\text{C}$ ) and 75-85 percent relative humidity were maintained throughout the rearing period.

## **2.2 *Ae. aegypti***

### **2.2.1 Rearing and Maintenance of *Ae. aegypti* mosquitoes**

A small plastic bowl (10cm diameter) was prepared by putting wet cotton and filter paper. This moist filter paper container was then placed in a cage of adults. The bowl was washed off followed by the removal of excess water. The egg paper was kept and remained for additional 24 hrs in cage. Thenafter the paper was removed and dried in air for 4 days followed by storage in a large sealed plastic container. Finally, the eggs were hatched in dechlorinated water at  $27^{\circ}\text{C}$  ( $80^{\circ}\text{F}$ ) temperature. Further, the colony was maintained at  $27^{\circ}\text{C}$  temperature, 75-85% relative humidity and 14:10 photoperiod.

### **2.2.2 Egg Production**

Blood fed females were kept in cage provided with a small water bowl cornered with Whatman filter paper for oviposition. During oviposition, it is important that no other open water sources must be present in the cage which can deter oviposition in the bowl (moist filter paper container). Under controlled laboratory condition mentioned above, oviposition begin on the second or third day after blood feeding and can continue for 1 or 2 more days. The same oviposition paper can be left in the cage for the duration of oviposition before collection. Egg counts were made daily from each group over a 4-6 days span. Average numbers of eggs laid were observed after 24, 48 and 72 hours.

### **2.2.3 Larvae**

The larvae generally hatch in 6-12 hrs. The larvae were counted by referring standard aliquot method and information was recorded. Approximately 67 larvae/cm<sup>2</sup> water surface having depth of 1.5 cm were introduced into the enamel bowl. The time for development of larvae varied from 8 to 25 days which was depending on temperature, type of food and density of larvae on a particular bowl. For the proper growth and development of larvae, the bowl water was changed on daily basis to check scumming. After collecting the pupae, these were transferred in cage for emergence. Approximately 100 pupae were kept in 30× 30× 30cm cage. For efficient egg laying the ratio of 2-3 females to 1 male was preferred. Female mosquito usually ingests a blood meal for egg laying. However, male adults require sugar solution for their development and hence 10% of sugar solution was provided to male mosquito. Sucrose and glucose in concentrations of 10% was prepared by 100 gm of household sugar dissolving in one litre water which appeared to provide the best growth and development. Soaked cotton balls were dipped in the sugar solution, and then placed on the top of the cage or cotton balls in a small plastic bowl directly kept inside the cage. The cotton balls were changed daily. During day a rabbit was kept every 48 hours in the adult cages in the iron frames to provide blood meal to the naturally mated adult female mosquitoes. Adult mosquitoes were maintained under the same environmental conditions as that larvae.

## **2.3 *Cx. quinquefasciatus***

### **2.3.1 Rearing and maintenance of *Cx. quinquefasciatus* Mosquitoes**

The larval culture of mosquito was maintained in laboratory at temperature 27°C, 75-85 percent relative humidity and 14:10 photoperiodically. The yeast were used for feeding the larvae. After feeding the larvae were converted into pupae. The pupae were collected from larval tray and were transferred to plastic bowl containing water. This plastic bowl was kept in cage to emerge into adult. The temperature, humidity and photoperiod were maintained same as for larvae. The cotton balls with 10% sugar solution were used for male adult mosquito feeding while female mosquitoes were fed with a rabbit blood. The males were provided with soaked cotton balls in the 10% sugar solution. The cotton ball was kept wet with sugar solution and changed regularly as mentioned above.

### **2.3.2 Egg production**

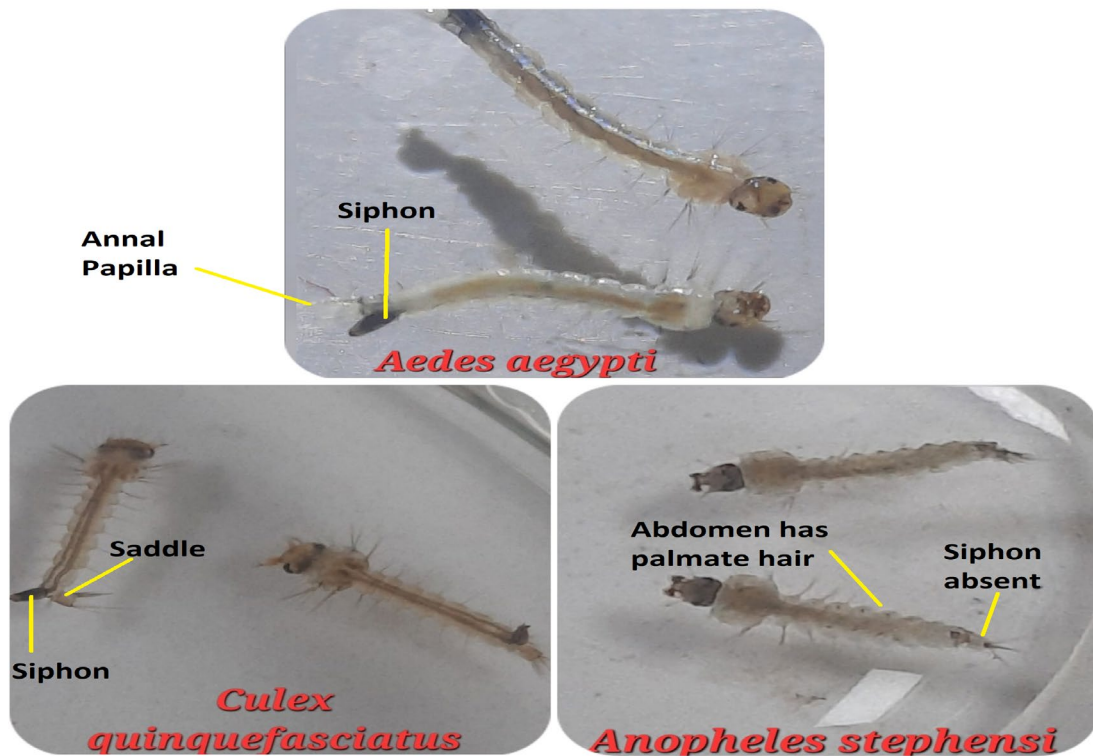
The gravid female mosquitoes land carefully on still water and lay eggs one by one, forming a raft that must remain on the water's surface to hatch. Collect egg rafts in the laboratory mosquito colony 3-7 days after blood feeding by placing a clear plastic bowl partially filled with tap water in the mosquito cage overnight. The egg rafts were gently transferred to a larval bowl filled with water and a pinch of larval food using water or a soft brush. The majority of eggs hatch into larvae within 48 hours.

### **2.3.3 Larvae**

Culex larvae feed voraciously during the aquatic stage. They frequently hang from the surface of the water, heads down, breathing through siphon tubes. Larvae shed (moult) their exoskeleton four times (4<sup>th</sup> instar stages), increasing in size with each moult. Larvae metamorphose into pupae after the fourth instar. Depending on temperature, crowding, and nutrition, the larval stage can last from 6 to 8 days. When these larvae reached the third instar in about four days, they were used in bioassays or reared to the fourth instar, pupae, and finally adults to maintain the running culture of the test species in the insectary, as previously discussed. (Figure. 3.1)



**Figure 3.1** Routine colony maintenance of mosquito vectors  
*(Ae. aegypti, Cx. quinquefasciatus and An. stephensi)*

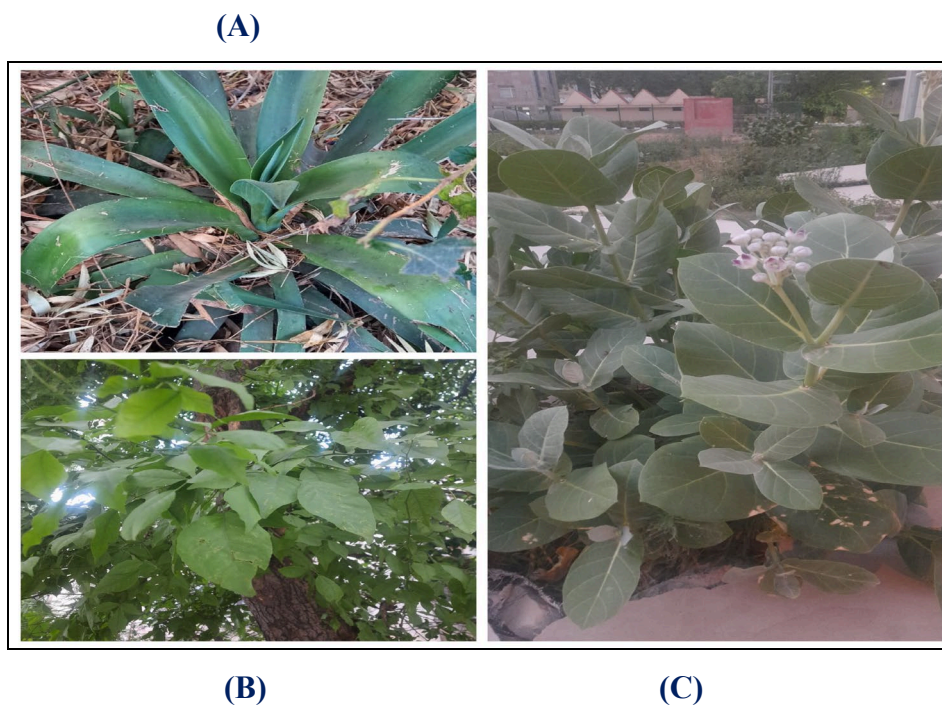


**Figure 3.2** Representation of selected mosquito vectors  
*(Ae. aegypti, Cx. quinquefasciatus and An. stephensi)*



### 2.3 Collection of Plants:

The fresh leaves of plants (*A. americana*, *A. marmelos*, *C. procera*) were collected from National Centre for Disease Control (NCDC) campus and ridge area (University of Delhi), Delhi (Figure 3.2 and Table 3.3)



**Figure 3.3 Representation of best selected plants**

**(A: *A. americana*, B: *A. marmelos*, C: *C. procera*)**

**Table 3.3 Detail of plants used in the study**

S.No.	Plant species	Common name	Part of the plants used	Collection area
1	<i>Calotropis procera</i>	Sodom apple	Leaves	NCDC campus, Delhi
2	<i>Aegle marmelos</i>	Bael	Leaves	NCDC Campus, Delhi
3	<i>Agave americana</i>	Century plant	Leaves	Ridge area, Delhi

**2.4 Preparation of leaf powder:** The plant leaves were cleaned with water and dried under shaded area at 27°C temperature for 15 days. After that the leaves were crushed in an electric grinder and powder was prepared.

**2.5 Preparation of leaf extracts:** Thirty grams of the powder was extracted with 250 ml of polar and non polar solvents (water, ethanol, hexane and acetone) for 8 hrs using Soxhlet apparatus at approx 70°C boiling temperature followed by filtration through a funnel with Whatman number 1 filter paper (Vogel 1978). The rotary vacuum evaporator was used to evaporate the leaf material and collected it into a vial then stored it in refrigerator for further use.

**2.6 Preparation of Stock Solution:** For the preparation of one percent stock solution, one gram of residue was dissolved in hundred mili litre of solvent used (same solvent was used in the extraction process).

#### **2.7 Preparation of various dilutions**

**For Bioassay:** The standard stock material was serially diluted according to WHO guidelines (2005). Triplicates of test volumes were prepared into final concentration.

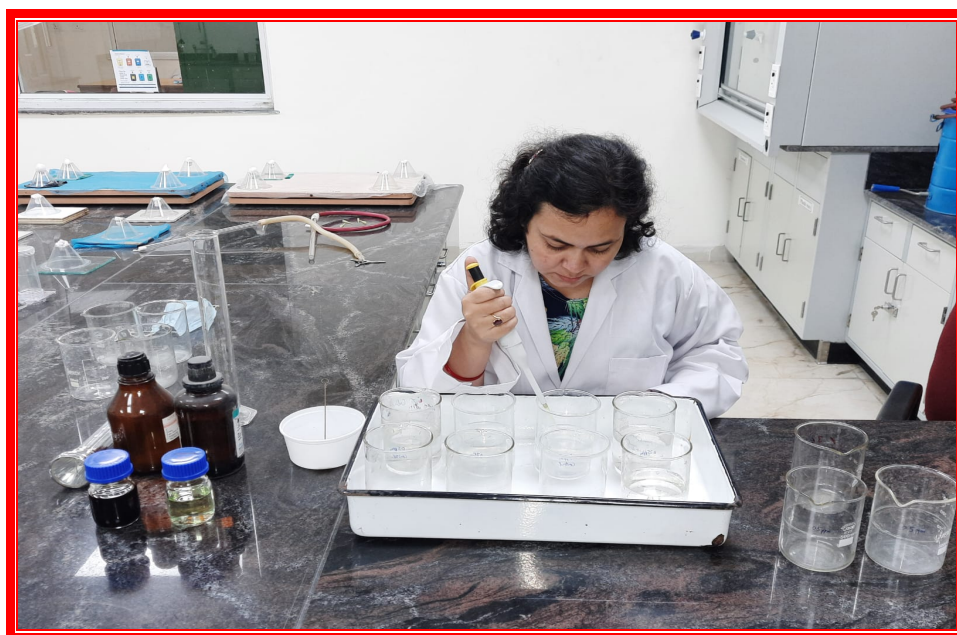
**2.8 Larvicidal Testing:** The larvicidal activity was carried out in NCDC's insecticide testing laboratory. According to WHO guidelines (2005), 25 numbers of late 3<sup>rd</sup> or early 4<sup>th</sup> instars larvae were kept in glass beaker of 500 ml capacity, containing 249 ml de-chlorinated water and one ml stock solution. Small, unhealthy or damaged larvae were removed. Yeast was given to larvae as food. Different concentrations taken to carry out the experimentation were ranging from 0.25ppm to 20ppm (Figure 3.3)

**2.9 Experimental Design:** Two to five experiments were performed at each concentration tested, with each experiment five replicates used for test and three for controls. Dechlorinated water was used as control to expose the larvae.

**2.10 Test for Larvicidal Activity:** After measuring larval mortality over this wide concentration range, mortality in 24 hours was determined at LC<sub>50</sub> and LC<sub>90</sub> values. Abbott's formula (Abbott's, 1925) was used to calculate control mortality.

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

**Statistical Analysis:** Probit analysis (Finney, 1971) was performed to calculate other statistics such as LC<sub>50</sub>, LC<sub>90</sub>, upper and lower confidence limits (UCL and LCL) at 95% confidence and chi-square values. Regression analysis was performed with software version SPSS 16.0. The significance criteria were P<0.05 for chi square test. The single way ANOVA method was used to analysis the variance using by Excel program.



**Figure 3.4 Experimental set up of larvicidal testing**

**3 High Performance Liquid Chromatography:** The optimization and development of HPLC were done at MRD Life Sciences Pvt. Ltd, Lucknow, Uttar Pradesh. Sunita (2012) and Mosihuzzaman (2008) reported methods for the elution of marmelosin which has low sensitivity and take long time to complete process, so some modification was done. 70 percent acetonitrile was used in mobile phase, flow rate 1ml/min and 247nm range of  $\lambda_{\text{max}}$  (maximum absorbance wavelength).

**Chromatographic conditions:**

Mobile phase: 70% Acetonitrile

Volume: 20 microliter

Flow rate: 1ml/min.

**Validation of Method:** The standard solutions were prepared with 10mg of marmelosin mixed with 10ml methanol (1mg/ml). Different standard solutions (1, 5, 10, 15,20,25,30 µg / ml) were prepared by the stock solutions in methanol (Panditrao, 2020). Limit of detection, quantitation, range, precision and accuracy was validated according to ICH (International Council on Harmonisation) guidelines.

#### **HPLC Quantitative Estimation of Marmelosin Biomarker Compound in *A. marmelos*:**

**Test solution:** The residues obtained from ethanol extracts of *A. marmelos* leaves were accurately weighed in triplicate and dissolved in HPLC grade methanol using 5 ml volumetric standard flasks filtered through 0.22 membrane filters before HPLC analysis.

**Standardization of Crude Extract:** Marmelosin content was collected from the plant *A. marmelos* leaves by the ethanolic extract. 1 mg of leaves were dissolved after weighing in ethanol. 30 mins left for sonicated this solution and then filtered through filter paper (Sunita 2012, Mosihuzzaman *et al.*, 2008). 10µg/ml solution was prepared and finally injected into HPLC.

**Assay of Herbal Preparation:** Twenty leaves tablets were weighed, equivalent to 10 mg of marmelosin was dipped in methanol and sonicated this solution for 30mins and then filtered by filter paper. Finally, diluted solution was injected into HPLC.

**4 Toxicological Testing:** For the testing, ten healthy specimens of *Channa punctatus* fish were taken with an average length and weight of  $1.2 \pm 0.10$  cm and  $1.09 \pm 0.9$  g, respectively. Morphologically, the body was elongated and cylindrical. Eyes are comparatively small and located anterior part of the head.

#### **4.1 Experimental Design:**

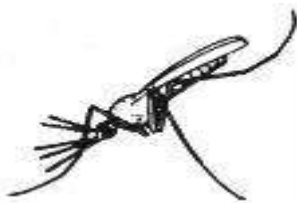
A glass aquarium was cleaned properly and filled with twenty litre of chlorine-free tap water and acclimated for two days with ten *Channa punctatus* fish. The *A. marmelos* ethanol extract concentration at 1.5 ppm was adjusted and remained in control when the fish were maintained in drug-free water. Mercury centigrade thermometer and a pH meter were used to measure the pH level (6.2 to 7.0) and temperature (58°C), respectively. Dissolved oxygen (DO) in the aquarium was monitored over the experimental period by dissolved oxygen meter.

#### 4.2 Histopathology:

Histopathological studies were done by following the standard protocols as described in the literature (Roberts, 2001). The sample preparation was done in ethanol for dehydration. Further, the samples were cleared by using xylene, and lastly soaked in liquid paraffin wax at 58° C temperature and finally kept in paraffin blocks. Sectioning of the samples was done by using a rotary microtome (leica RM2255) which was set to section the samples at 6 µm in size followed by staining with Hematoxylin and Eosin with Microm HMS7. The stained sections were observed under the light microscope (Olympus CX21). (Figure 3.4).



**Figure 3.5 Demonstration of *Channa punctatus* fish for Histopathological testing**



## CHAPTER 4 RESULT & DISCUSSION

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The results and discussion have been explored under the following headings:

- 4.1. Computational study of plants against *Ae. aegypti*.
- 4.2. Computational study of plants against *Cx. quinquefasciatus*.
- 4.3. Computational study of plants against *An. stephensi*.
- 4.4. Larvicidal activity of *C. procera* leaves extract against *Ae. aegypti*.
- 4.5. Larvicidal activity of *C. procera* leaves extract against *Cx. quinquefasciatus*.
- 4.6. Larvicidal activity of *C. procera* leaves extract against *An. stephensi*.
- 4.7. Larvicidal activity of *A. marmelos* leaves extract against *Ae. aegypti*.
- 4.8. Larvicidal activity of *A. marmelos* leaves extract against *Cx. quinquefasciatus*.
- 4.9. Larvicidal activity of *A. marmelos* leaves extract against *An. stephensi*.
- 4.10. Larvicidal activity of *A. americana* leaves extract against *Ae. aegypti*.
- 4.11. Larvicidal activity of *A. americana* leaves extract against *Cx. quinquefasciatus*.
- 4.12. Larvicidal activity of *A. americana* leaves extract against *An. stephensi*.
- 4.13. Quantitative analysis of an ethanolic extract of *A. marmelos*.
- 4.14. Toxicological analysis of an ethanolic extract of *A. marmelos*.

#### 4.1 Computational study of plants against *Ae. aegypti*.

The mol files of the ligands and three-dimensional structure of the secondary metabolites from *Ae. aegypti* were put as input in CB-Dock server for blind docking. The CB-Dock server provides all information regarding the activity of secondary metabolites. The activities of the secondary metabolites and compounds presented in Table 4.1. In the present study, phyto constituents of different plants have revealed a significant level of docking score and interaction energies against *Ae. aegypti* proteins 6P2E, 6DU6 and 5V13 (Table 4.1). The highest interaction energy score was found to be  $-10.4$  Kcal/ mol against 5V13 protein of *Ae. aegypti*. The highest interaction energy score was found by the phytoconstituents of *A. marmelos* followed by *A. americana* and *C. procera* respectively.

In addition, the 6P2E protein of *Ae. aegypti* belongs to OBP (Odorant-binding proteins) family of proteins, which has been specifically linked to controlling certain feeding habits. The 6P2E protein is distinctive because it is expressed in a variety of chemosensory tissues, such as the antenna, the females' proboscis, the male reproductive glands, the salivary glands and thoracic spiracles, where it is transported to the females during mating (Shaalán *et al.*, 2005). Interestingly, it was found that 6P2E's expression in the salivary glands is upregulated in response to Dengue virus (DENV) infection when combined with other chemosensory genes, and that knocking down 6P2E using dsRNA methods reduced blood feeding habits (Singh *et al.*, 2005). Beta amyirin (pentacyclic triterpenoid) was discovered to have a high affinity for the mosquito OBP and to engage with it, suppressing the mosquito population and minimizing man-vector contact. The investigation of in silico docking studies of mosquito repellent chemicals from *Hyptis suaveolens* found a similar finding. (Sukumar *et al.*, 1991).

Additionally, 6DU6 is PK1 (Pyruvate kinase), mosquitoes of the genus *Ae. aegypti* like to feed on human blood and typically fly only a short distance in order to remain near to their human victims (Torres *et al.*, 2015). *Ae. aegypti* can utilise the amino acid proline to power flight in addition to carbohydrate sources by way of the proline-alanine cycle (Panditrao *et al.*, 2020). Proline acts as a shuttle in this cycle, transporting acetyl units between the flying muscles and the fat body. Alanine aminotransferase, an enzyme engaged in amino acid and ammonia metabolic in *Ae. aegypti*, can use the pyruvate produced through many processes, including the decarboxylation of malate or because of PK (6DU6), as a substrate (Wang *et*

*al.*, 2012). Female mosquitoes as well as other blood-feeding species of the dipteran suborder Nematocera have saliva that contains proteins from the mosquito odorant-binding protein (OBP) family known as 5V13 (D7). The suppresser of 5V13 protein can be good mosquito repellent (Singanan *et al.*, 2007). The 5V13 protein from *Ae. aegypti* has the greatest interaction energy score, which was found to be  $-10.4 \text{ kcal mol}^{-1}$ . The phytoconstituents of *A. marmelos*, followed by *A. americana* and *C. procera*, had the greatest interaction energy score.



**Table 4.1:** Tabular display of docking score of the *Aedes* larval essential proteins with phyto constituents

<b>Name of Phytoconstitute</b>	<b>ChemSpider ID of Phyto constituents</b>	<b>Interaction energy (kcal/mol) against 6P2E protein</b>	<b>Interaction energy (kcal/mol) against 6DU6 protein</b>	<b>Interaction energy (kcal/mol) against 5V13 protein</b>
<b><i>Cannabis sativa</i></b>				
$\gamma$ -Terpinene	7181	-6.7	-7.1	-7.4
$\beta$ -Terpinene	60205	-6.8	-7	-7.4
Linalool	60523	-6.0	-5.7	-6.8
Linalool	391430	-6.0	-6.0	-6.8
trans-Anethole	553166	-6.7	-6.8	-7.3
Linalool	1266019	-6.6	-6.9	-7.6
Linalool	13849981	-6.0	-6.1	-6.6
<b><i>Calotropis procera</i></b>				
Urs -19- en -3 yl acetate	164675	-6.8	-8.6	-8.5
Sitosterol	192962	-6.7	-9.4	-8.2
Stigmasterol	4444352	-7.7	-9.3	-8.5
Multiflorenol	32700975	-7.5	-8.7	-8.4
IN00242	64870692	-7.9	-8.6	-10.2
<b><i>Datura stramonium</i></b>				

2-chloro-4-aminotoluene-5-Sulfonic Acid	6669	-6.2	-6.9	-7
Acetoacet-o-chloranilide	6889	-6.9	-7.4	-7.5
2-chloro-4-aminotoluene	6985	-6.0	-5.8	-6.3
Methyldiphenylamine	10627	-7.3	-8.3	-8.4
N,4-dimethylaniline	11665	-6.1	-5.7	-6.2
2-methylaniline	12723	-8.3	-8.7	-9
Diazepam related compound A	13323	-7.9	-7.9	-8.8
3-methyldiphenylamine	13910	-7.6	-8.6	-8.9
4-bromo-m-toluidine	21844	-6.1	-5.9	-6.3
$\alpha$ -Solanine	28033	-8.3	-8	-10.1
2-ethyl-6-methylaniline	30109	-6.2	-6.4	-6.8
2-amino-4-methylbenzoic acid	67854	-6.6	-6.5	-7
3-amino-p-toluic acid	68093	-6.7	-6.3	-6.9
3-methyl-4-aminobenzoicacid	68122	-6.8	-6.4	-7.2
Zr d1 bvq	68734	-6	-6.4	-7.1
5-amino-2-methylbenzenesulfonamide	205478	-6.3	-6.4	-7.1

4-amino-2-methylbenzoic acid	211192	-6.4	-6.3	-6.8
Methyl 3-amino-p-toluate	299371	-6.3	-6.5	-6.9
$\alpha$ -chaconine	391274	-8.1	-8	-9.1
4-amino-3,5-dichlorobenzonitrile	455957	-6	-6.2	-6.3
Methyl 4-amino-3-methylbenzoate	2018453	-6.4	-6.6	-7
5-bromo-4-fluoro-2-methylaniline	2062930	-6.0	-6.2	-6.7
6-amino-2-methylnicotinonitrile	2073554	-6.1	-6.2	-6.3
2-amino-4-methylbenzonitrile	2079989	-6.2	-6.4	-6.9
4-nitrotoluene	13863774	-6.4	-6.2	-6.6
Methyl-3-8-methyl-8-azabicyclo octane-2-carboxylate	4937726	-6.6	-7.6	-7.9
4-amino-3-methylbenzonitrile	5373889	-6.3	-6.3	-6.7
3-amino-p-tolunitrile	5379440	-6.3	-6.3	-6.7
Cocaine	10194104	-7.6	-7.6	-8.4
M-cresidine	21106028	-6.1	-5.8	-6.2
2-methyl 3-phenyl-8-methyl-8-azabicyclo	23202611	-6.8	-7.4	-7.8

octane-2,3-dicarboxylate				
<i>Aegle marmelos</i>				
2,3-pinane-1,2-diol	55886	-6.2	-6.4	-7.1
Ethyl (3,4-dichlorophenyl) acetate	149921	-7.0	-7.4	-8
Ethyl {2 - [(4 - chlorophenyl) amino]-1,3-thiazol-4-yl} acetate	963724	-6.7	-7	-9.4
Ethyl {2 - [(4-fluorophenyl) amino] 1,3-thiazol-4-yl} acetate	2050189	-6.7	-6.8	-8.6
Ethyl {2 - [(4-methyl phenyl) amino]-1,3-thiazol-4-yl} acetate	3953401	-7.0	-7.1	-9.4
Ethyl (2E)-chloro[(3,5-dichlorophenyl)hydrazono] Acetate	4590540	-6.8	-6.9	-7.7
Ethyl (2E)-chloro[(2-chlorophenyl)hydrazono] acetate	4728198	-6.9	-7.5	-7.4
Ethyl chloro((4-nitrophenyl)hydrazono) acetate	4736864	-7.0	-7.3	-8.2
Ethyl (2E)-[(4-bromophenyl)hydrazono]	4757948	-6.4	-6.5	-8.1

o](chloro)acetate				
Ethyl (2E)-chloro[(4-chlorophenyl)hydrazono]acetate	4831380	-6.6	-6.7	-8
Ethyl (2E)-chloro[(2-methylphenyl)hydrazono]acetate	4838953	-7.1	-7.7	-7.6
Ethyl (2E)-chloro[(2-fluorophenyl)hydrazono]acetate	4838956	-7.0	-7.4	-7.6
Ethyl (2E)-[(4-tert-butylphenyl)hydrazono](chloro)acetate	4838958	-6.8	-7.0	-9.4
Ethyl {2- [(3 - chlorophenyl) amino] - 1,3-thiazol- 4 -yl} acetate	5509850	-6.7	-7.0	-8.7
Ethyl (2E)-amino[(2,4-dichlorophenyl)hydrazono]acetate	9812399	-6.8	-6.9	-8
Ethyl (3,4-difluorophenyl)(difluoro)acetate	10325010	-7.2	-7.5	-8.4
Ethyl (4-chlorophenyl)(difluoro)acetate	14010415	-6.9	-7.4	-8
Ethyl {2-[(2-fluorophenyl)amino]-	16783979	-6.7	-7.0	-8.5

1,3-thiazol-4-yl} acetate				
4-[2-(4-[(3-Chloro-4-methoxyphenyl)carbamoyl](hydroxy)amino)-5,5-dimethyl-2-thioxo-1,3-thiazolidin-3-yl)ethyl]morpholin-4-ium acetate	21343927	-2.7	-7.9	-8.1
Ethyl difluoro(3-fluoro-4-methoxyphenyl)acetate	21391591	-7.4	-7.2	-8.1
Ethyl difluoro(3-methoxyphenyl)acetate	21391594	-7.2	-7.2	-7.9
Ethyl (3,5-difluorophenyl)(difluoro) acetate	21391601	-7.2	-7.4	-8.5
Ethyl (4-butylphenyl)(difluoro) acetate	21391603	-7.4	-7.6	-9.1
Ethyl (4-tert-butylphenyl)(difluoro) acetate	21391604	-6.9	-7.4	-9.5
Ethyl (3,4-dimethylphenyl)(difluoro) acetate	21391607	-7.4	-7.8	-8.3
Ethyl (3-chloro-4-fluorophenyl)(difluoro)	21391608	-7.3	-7.6	-8.3

acetate				
Ethyl difluoro(3-fluoro-4-methylphenyl)acetate	21391611	-7.4	-7.9	-8.4
Ethyl (3,4-dimethoxyphenyl)(difluoro)acetate	21391612	-6.6	-7.2	-7.5
Ethyl difluoro(4-isopropylphenyl)acetate	21391615	-7.5	-7.7	-9.1
Ethyl difluoro(4-methylphenyl)acetate	21391616	-7.1	-7.6	-8.3
michaolide G	28638995	-6.1	-7.5	-8.4
4-(2-Carboxy-4-{{[(4,5-dimethoxy-3-oxo-1,3-dihydro-2-benzofuran-1-yl)acetyl]amino}phenyl}-1-methylpiperazin-1-ium acetate	32513287	-2.7	-8.0	-8.0
(1Z,2Z)-N-(2-Hydroxy-2-{{4-[(3-methyl-2-buten-1-yl)oxy]phenyl}ethyl)-3-phenyl-2-propenimidic acid	35013158	-7.7	-7.9	-10.3
IN00216	6502	-6.3	-7.0	-7.0
Ammijin	187477	-8.1	-8.7	-10.4
Rutin	4444362	-7.9	-9.1	-9.7

Sitosterol	192962	-7.5	-7.6	-7.9
Citronellal	7506	-6.0	-5.3	-6.6
Eugenol	13876103	-6.5	-5.5	-7.1
<i>Agave Americana</i>				
(2S, 3S, 4S, 5R, 6R) -6- {[(2S, 3S, 4S, 5R) -2- ([(2R, 3S, 4S, 5R) -3, 4 – di hydroxyl -2, 5- bis (Hydroxy methyl) tetrahydro -2- furanyl]oxy} methyl)- 3,4-dihydroxy-5- (hydroxymethyl)tetrahy dro- 2 -furanyl] oxy} Tetra hydro- 2H – pyran -2 , 3, 4, 5-tetrol	52082957	-6.0	-7.2	-8.6
Isoflavone	65255	-8.4	-9.4	-9.7
Spirostan -3- yl 2 - O - [2- (hexopyranosyloxy) - 3, 4, 5, 6- tetrahydroxycyclohexyl] - 4 – O – pentopyranosylhexopyr anoside	153288	-7.9	-8	-10.3
1-O- beta -D- Fructofuranosyl- beta - D- fructo furanose	388643	-5.6	-7.5	-7.5
1-O- beta -D-Fructo furanosyl- beta -D-	9182610	-7.5	-8.0	-9.2



fructofuranose				
(2S, 3S, 4S, 5R, 6R) -6- {[ (2S, 3S, 4S, 5R) -2-({ (2R, 3S, 4S, 5R) -3, 4- di hydroxyl - 2, 5-bis (Hydroxyl methyl) tetrahydro - 2 -furanyl] oxy} methyl) - 3, 4 - Di hydroxyl - 5 -(Hydroxyl methyl) tetrahydro - 2 - furanyl] oxy} Tetra hydro -2H - pyran - 2, 3, 4, 5-tetrol	52082957	-6.0	-7.1	-8.6
<i>Achyranthus aspera</i>				
12-o- tetradecanoylphorbol- 13-acetate	25977	-7.4	-7.6	-7.2
Dihexadecyl ketone	29298	-5.5	-5.0	-5.2
16-tritriacontanone - 8- pentatriacontanone	213083	-8.8	-5.4	-8.6
16-tritriacontanone	213085	-5.8	-5.1	-5.0
Aconitic acid	392201	-5	-6.1	-6.0
3-tritriacontanone	474943	-5.4	-5.4	-8.6
2-tritriacontanone	476843	-5.6	-5.1	-5.0
24,25- bis(methylsulfanyl)-2- tritriacontanone	550318	-5.4	-5.4	-6.6

MFCD00026601	4366538	-6	-5.1	-5.0
12-O-Tetradecanoylphorbol-13-acetate	8651284	-6	-5.2	-5.6
Glucopyranoside	23253896	-5.8	-5.1	-5.0
$\alpha$ -D-Galp-(1->3)- $\beta$ -D-Galf - (1->3) - $\alpha$ -D-Manp- (1->3)- $\alpha$ -D-Manp - (1->4) – $\alpha$ -D- Glcp N - (1->6) – 1 D – myo – inositol	26332395	-7.2	-7.2	-8.3
10-tritriacontanone	59696654	-5.4	-5.2	-5.0
12-tritriacontanone	59696655	-6	-5.3	-8.5
14-tritriacontanone	59696656	-5.7	-5.3	-5.2
8-tritriacontanone	59696657	-5.9	-5.1	-6.6
18-hydroxy-16-tritriacontanone	35014819	-5.9	-5.2	-5.0
28-hydroxy-6-methyl-5-tritriacontanone	35013894	-5.4	-5.2	-5.6
<i>Eichhornia crassipes</i>				
Shikimic acid	9161960	-5.8	-7.0	-7.5
Shikimic acid	8412	-5.7	-6.0	-5.9
1,1-Diphenyl-2-(2,4,6-trinitrophenyl)diazene	15122	-6.8	-8.0	-8.8
DPPH	66953	-7	-7.9	-8.7

Sitosterol	192962	-7.5	-8.7	-7.7
DPPH	2016757	-6.7	-8	-8.7
Stigmasterol	4444352	-7.4	-9.6	-7.8
<i>Mentha piperita</i>				
Piperitenone Oxide	390924	-7.3	-6.0	-7.4
Ot0175110	10756	-6.6	-5.6	-7.2
Piperitenone Oxide	55800	-6.7	-6.3	-7.4
<i>Catharanthus roseus</i>				
Urs-12-en-3-yl acetate	259299	-7.0	-8.4	-8.0
Oleanolic acid	10062	-7.1	-8.3	-8.3
$\beta$ -Amyrin acetate	83201	-7.2	-8.8	-8.3
266N1630AL	83811	-7.5	-8.2	-8.6
<i>Allium ampeloprasum</i>				
Ethane-1,2-d2	24532533	-1.8	-2.0	-1.9
Ethane-1,1-d2	124782	-1.8	-1.9	-2.0
Limonene	389747	-6.7	-5.8	-7.4
Pinene	389794	-6.8	-5.3	-7.5
$\alpha$ -Pinene	389795	-6.9	-5.5	-7.4
$\alpha$ -Farnesene	4444849	-7.3	-6.2	-8.7
$\beta$ -Farnesene	4444850	-7.3	-6.1	-8.4
$\beta$ -Pinene	8466294	-6.8	-1.9	-2.0
Terpineol	13850142	-6.4	-5.8	-7.4

βeta- D –gluco pyranoside, 2, 4-Di hydroxyl -6- (2-(4- Hydroxy phenyl) ethenyl) phenyl	19026512	-6.8	-5.3	-7.5
Ethane-d5	21170395	-1.8	-5.5	-7.4
Carbon tetrachloride	5730	-3.6	-6.2	-8.7
α-Pinene	6402	-6.9	-5.5	-7.4
XU2150000	7175	-6.3	-1.9	-2.0
γ-Terpinene	7181	-6.7	-5.8	-7.4
MM1997800	11110	-4.2	-5.3	-7.5
4, 5- epoxy - 4, 11, 11- Tri methyl – 8 – Methylene bicycle (7.2.0) undecane	13711	-6.7	-5.5	-7.4
β-Pinene	14198	-6.8	-6.2	-5.7
Dimethyl N-Hydroxy Methyl Carbamoyle Thylphos Phonate	27833	-4.9	-1.9	-2.0
β-Myrcene	28993	-5.9	-5.8	-5.4
Ethane	120830	-1.8	-5.3	-7.5
α-Pinene	74205	-6.9	-5.1	-7.4
Dimethyltetrasulfane	72121	-2.6	-6.2	-6.7
Phenosafuranine	59155	-7.6	-7.3	-7.2
<i>Aloe vera</i>				

Salicylic acid	331	-5.9	-4.0	-5.6
Disodium (1Z)-N-[(7Z)-8-oxo-7-(phenylhydrazono)-6-sulfo-4-sulfonato-7,8-dihydro-1-naphthalenyl]ethanimidate	59696683	-7.9	-9.2	-9.2
2-Hydroxybenzoic acid - 2-(1-piperazinyl)ethanamine (1:1)	57461842	-8.2	-7.4	-9.7
2-Hydroxybenzoic acid - 1-butyl-1H-imidazole (1:1)	21165422	-9.3	-8.9	-10.1
(1 $\beta$ ,3 $\beta$ ,25R)-3-Hydroxyspirost-5-en-1-yl $\beta$ -D-Glucopyranosyl - (1->2)- [ $\beta$ -D - xylo pyranosyl- (1->3)] -6 - De oxy- $\beta$ -D - galactopyranoside	9182610	-7.4	-9.8	-9.0
(1R,2S)-Ethyl 1-(Boc-amino)-2-vinylcyclopropanecarboxylate	8834028	-5.7	-5.6	-5.0
Imidazole salicylate	34333	-7.2	-6.9	-6.5

Caffeine salicylate	57699	-9.8	-9.9	10.2
2-Hydroxybenzoic acid - 8- methyl -8-aza bi cyclo [3.2.1] oct- 3 - yl 3- hydroxy - 2 - phenylpropanoate (1:1)	58210	-7.4	-8.9	-9.3
Antipyrine Salicylate	91924	-9.2	-9.2	-9.2
4-[(4-aminophenyl)methyl]aniline; 2-(chloromethyl)oxirane; 2-hydroxybenzoic acid; 4-[1-(4-hydroxyphenyl)-1-methyl-ethyl]phenol; phenylmethanol	150627	-10.1	-9.1	-9.5
Formaldehyde; 2-hydroxybenzoic acid; 6-phenyl-1,3,5-triazine-2,4-diamine	152424	-9.9	-9.9	-10.0
L-Lysine - 2-hydroxybenzoic acid (1:1)	2338582	-9.3	-8.9	-10.0
Aminopyrine salicylate	2340811	-9.7	-9.5	-9.0
Aminopyrine salicylate	4450242	-6.7	-6.9	-8.0

Nicotine Salicylate	7972119	-8.5	-8.0	-9.8
<b><i>Lantana camara</i></b>				
Oleanonic acid	10194990	-7.2	-8.3	-8.5
DPPH	2016757	-7.0	-8.0	-8.7
(E)-Aconitic Acid	392201	-5.1	-6.1	-6.9
DPPH	66953	-6.9	-8.0	-8.7
1,1-Diphenyl-2-(2,4,6-trinitrophenyl)diazonium	15122	-6.8	-8.1	-8.8
<b><i>Ficus bengalensis</i></b>				
(25E)-25-Tetratriaconten-2-one	4517307	-5.6	-5.1	-5.0
Inositol	10239179	-5.2	-6.9	-6.5
(1R, 2S, 3S, 4R) -1, 2, 3, 4, 5, 6 –cyclo hexane hexol	32696449	-5.0	-8.6	6.3
<b><i>Eucalyptus camaldulensis</i></b>				
$\beta$ -Terpinene	60205	-6.7	-7.9	-6.3
(1s, 5s) – 4 – isopropyl - 1- Methyl - 6- Oxa bi cyclo [3.1.1] heptanes	59696307	-6.5	-9.2	-9.2
(1r, 6r) - 1, 3, 3 –tri	58837155	-7.0	-9.1	-9.5

methyl – 2 – Oxa bi cyclo [2.2.2] octan -6- ol				
(1R,6S)-1,3,3- Trimethyl-2-Oxa bi cyclo [2. 2. 2] octan- 6 -ol	58829897	-6.9	-9.9	-8.9
1, 3, 3-tri methyl – 2 – Oxa bi cyclo [2. 2. 2] Octan - 6- ol - acetic acid (1:1)	57465927	-8.0	-8.9	-9.0
1,3,3- tri methyl – 2 – Oxa bi cyclo [2. 2. 2] Octane	57257156	-7.0	-9.5	-8.0
(4r, 6s) - 1, 3, 3 –tri methyl – 2 –Oxa bi cyclo [2. 2. 2] Octan – 6 - ol	57256935	-6.9	-6.9	-8.0
(1R, 4R)- 1, 3, 3 –tri methyl – 2 –Oxa bi cyclo [2. 2. 2] Octane	31045960	-7.0	-8.0	-9.8
(1S,4R,6S)-3,3- Dimethyl-2- oxabicyclo[2.2.2]octan- 6-ol	30783268	-6.2	-8.3	-8.5
OS9274000	28295867	-6.4	-8.0	-8.7



Cyclohexanol - 1, 3, 3 – Tri methyl – 2 –Oxa bi cyclo [2. 2. 2] Octane (1:1)	24592609	-10.3	-5.6	-5.0
1- iso propyl – 4 – methyl – 7 –Oxa bi cyclo [2. 2. 1] Heptanes	9702	-6.4	-6.9	-6.5
$\gamma$ -Terpinene	7181	-6.7	-9.9	12.3
( $\pm$ )- $\alpha$ -Pinene	6402	-6.9	-8.9	-9.3
( $\pm$ )-Eucalyptol	2656	-7.0	-9.2	-9.2
(1s,4s)-Eucalyptol	21111689	-7.0	-10.1	-9.5
Globulol	16737082	-7.4	-9.9	-9.9
2-exo-hydroxy-1,4- cineole	10260743	-6.4	-8.9	-9.0
Sesquicineole	10250069	-7.7	-9.5	-9.0
(+)-endo-2-acetoxy-1,8- cineole	9393167	-7.4	-6.9	-8.0
1,3,3- tri methyl – 2 – Oxa bi cyclo [2. 2. 2] Oct -6-yl Acetate	152579	-6.9	-8.0	-9.8
$\alpha$ -pinene oxide	82629	-6.8	-8.3	-8.5
(+)- $\alpha$ -Pinene	74205	-6.9	-8.0	-8.7

1,3,3- tri methyl – 2 – Oxa bi cyclo [2. 2. 2] Octan – 6 – ol	461626	-7.1	-5.6	-5.0
2-exo-hydroxy-1,8- cineole	5256723	-6.9	-6.9	-6.5
2-endo-hydroxy-1,8- cineole	5256807	-7.0	-9.9	10.1
Dehydro-1,8-cineole	456244	-7.0	-8.9	-9.3
(-)- $\alpha$ -Pinene	389795	-6.9	-9.2	-9.2
<b><i>Ocimum sanctum</i></b>				
Methylamine	6089	-1.7	-18.1	-18.5
(1s, 3r, 5r, 6r, 8r, 10r, 11r, 13r, 15r, 16r, 18r, 20r, 21r, 23r, 25r, 26r, 28r, 30r, 31s, 33r, 35r, 36r, 37s, 40r, 41s, 42r, 43s, 44r, 45s, 46r, 47s, 48r, 49s) -5, 10, 15, 20, 25, 30, 35 –Heptakis (hydroxymethyl) -37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49-Dode came thoxy -2, 4, 7, 9, 12, 14, 17, 19, 22,24,27,29,32,34- tetradecaoxaocyclo nonatetracontane-36,38-	64849692	-4.9	-10.0	-10.1

diol (non-preferred name)				
(1s, 3r, 5r, 6r, 8r, 10r, 11r, 13r, 15r, 16r, 18r, 2	57620332	-4.7	-8.9	-11.0
(4R,5S,6S,7S,4'R,5'S,6'S,7'S)-2,2'-[Oxybis(methylene)]bis(3,4,5,6,7,8-hexahydroxyoctanenitrile)	57488057	-5.5	-9.5	-10.0
2- o - (2-hydroxy ethyl) - 4 - o - [2-o- (2-Hydroxy propyl) hexopyranosyl]hexopyranose	57487300	-5.6	-6.9	-8.0
3-(Allyloxy)-1-propene - $\alpha$ -D-fructofuranosyl $\alpha$ -D-glucopyranoside (1:1)	57486960	-7.5	-8.0	-9.8
2,4,5-T Dimethylamine	21418	-7.8	-8.3	-8.5
2,4,5-T-trolamine	18522	-7.4	-8.0	-8.7
MFCD00027307	17558	-5.4	-5.6	-5.0
2,4,5-T-Triethylammonium	15358	-7.3	-6.9	-6.5

Decane	14840	-5.3	-9.9	10.1
Bindone	14812	-9.6	-8.9	-9.3
2-Methyldodecane	14535	-6.1	-9.2	-9.2
2-Methyltetradecane	14533	-5.7	-9.1	-9.5
Zinc Propionate	10716	-5.9	-9.9	-8.9
Hexanitrodiphenylamine	8258	-6.2	-8.9	-8.0
Methyl- $\beta$ -cyclodextrin	26324307	-4.7	-10.1	-10.0
(3Z)-3-{[4-Amino-5-(3,4,5-trimethoxybenzyl)-2-pyrimidinyl]imino}-5-chloro-1-[4-(4-nitrophenyl)-1-piperazinyl] Methyl}-1,3-Dihydro-2H-Indol-2-one	32763347	-9.0	-6.9	-8.0
3'-O-(hydroxyethyl)rutoside	32702185	-7.7	-8.0	-9.8
3',7-di-O-(hydroxyethyl)rutoside	32701307	-7.4	-8.3	-8.5
3',4',5-tri-O-(hydroxyethyl)rutoside	32700275	-7.0	-8.0	-8.7

5,4'-di-O-(hydroxyethyl)rutoside	32700195	-7.7	-5.6	-5.0
5,3'-di-O-(hydroxyethyl)rutoside	32700093	-7.5	-6.9	-6.5
3',5,7-tri-O-(hydroxyethyl)rutoside	32700032	-7.0	-9.9	9.3
4',7-di-O-(hydroxyethyl)rutoside	32699299	-7.1	-8.9	-9.3
3',4'-di-O-(hydroxyethyl)rutoside	32699278	-7.0	-9.2	-9.2
5,7-di-O-(hydroxyethyl)rutoside	32699175	-6.9	-9.1	-9.5
5-O-(hydroxyethyl)rutoside	32698032	-7.4	-10.0	-9.9
4'-O-(hydroxyethyl)rutoside	32697781	-8.3	-8.9	-9.0
(2-Hydroxyethyl)- $\beta$ -cyclodextrin	30784495	-4.8	-10.1	-10.0
2-Methyldecane	21896	-5.6	-6.9	-8.0
N,N-Dimethyl-19-tetracontanamine	24764583	-5.6	-8.0	-9.8
Dimethyltridecylamine	34100	-5.5	-8.3	-8.5

Oxide				
2,2',3,4',5,6'- Hexabromodiphenyl ether	34359	-5.2	-8.0	-8.7
2,3,4,4'- Tetrabromodiphenyl Ether	35182	-7.5	-5.6	-5.0
2,2',4,4',5- Pentachlorodiphenyl ether	39386	-7.8	-6.9	-6.5
(2,4,5- Trichlorophenoxy)aceti c acid - N,N- dimethylmethanamine (1:1)	55326	-6.4	-9.9	10.1
(2,4,5- Trichlorophenoxy)aceti c acid - 1-amino-2- propanol (1:1)	56184	-7.5	-8.9	-9.3
Ethylenebis(chlorodime thylsilane)	75384	-5.5	-9.2	-9.2
2-Ethyl-N-(3- ethylhexyl)-N-methyl- 1-hexanamine	77683	-5.6	-8.1	-8.5
13-Ethoxy-1- tridecanamine -	84318	-1	-9.9	-9.9

methane (1:1)				
2,2',4,4'- Tetrabromodiphenyl ether	85876	-6.8	-8.9	-9.0
NDA	87023	-7.5	-8.5	-8.0
MFCD00037841	89900	-7.4	-6.9	-8.0
3-Methyl-1-hexanamine	93368	-4.9	-8.0	-9.8
Dipalmithyl methylamine	95188	-5.6	-8.3	-8.5
N,N-Dipentadecyl-1- hexadecanamine	95189	-5.6	-8.0	-8.7
1-Nonadecanamine	95814	-5.8	-5.6	-5.0
12-Ethoxy-3-methyl-4- (3-methyl-2-butanyl)-1- dodecanamine	98337	-6.2	-6.9	-6.5
5-Methyl-N-(9- methyldodecyl)-1- dodecanamine	98401	-6.0	-9.9	10.1
6-Methyl-1-decanamine	98431	-5.8	-8.9	-9.3
6-Methyl-1- tetradecanamine	98432	-6	-9.2	-9.2
N-Pentadecyl-1-	98543	-5.0	-8.1	-8.5

pentadecanamine				
2-Propyn-1-ol - 2-(chloromethyl)oxirane (1:1)	98952	-5.6	-8.9	-9.9
N-Methyl-N-pentadecyl-1-pentadecanamine	98997	-6.2	-8.9	-9.0
Methyldimyrystylamine	99141	-6.3	-10.0	-10.1
14-Ethoxy-13,13-dimethyl-1-tetradecanamine	100055	-5.1	-6.9	-8.0
1-[(2-Methyl-2-propanyl)oxy]pentadecanamine	101814	-5.3	-8.0	-9.8
37,39,41,43,45,47,49-Heptamethoxy-5,10,15,20,25,30,35-heptakis(methoxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29,32,34-tetradecaoxaocyclo[3.1.2.2.2 <sup>3,6</sup> .2 <sup>8,11</sup> .2 <sup>13,16</sup> .2 <sup>18,21</sup> .2 <sup>23,26</sup> .2 <sup>28,31</sup> ]nonatetracontane-36,38,40,42,44,46,48-heptol	108942	-5.0	-8.3	-8.5



(8E,14E)-8,14-Hexadecadien-1-amine	4942209	-6.1	-8.0	-8.7
Tetrabromodiphenyl ether	17215736	-5.6	-5.0	-5.1
10,10-Dimethyl-1-undecanamine	17215523	-6.9	-6.5	-6.0
{3-[4-(Hydroxymethyl)phenoxy]phenyl}methanol	9543124	-6.0	-6.0	-6.2
4',5,7-tri-O-(?-hydroxyethyl)rutoside	8254267	-6.2	-6.2	-5.9
Dimethylpentadecylamine oxide	8035971	-5.9	-5.9	-5.8
Monoxerutin	8028296	-5.8	-5.8	-6.0
(9E,16E)-1-Ethoxy-9,16-hexatriacontadiene	4952962	-6.0	-6.0	-4.9
SODIUM PROPYLENE GLYCOL SULFONATE	4957982	-4.9	-4.9	-5.0
2-Butyne-1,4-diol - 1-chloro-2-(2-chloroethoxy)ethane (1:1)	4958174	-5.0	-5.0	-6.2

(7E)-N,N-Dimethyl-7-hexadecen-1-amine	4952959	-6.1	-6.2	-5.8
(1E)-1,4-Pentadien-1-amine	4942238	-5.8	-5.8	-5.3
1,1,1,2,2-Pentafluoro-2-iodoethane - tetrafluoroethene (1:1)	141844	-8.1	-8.5	-5.2
(9E)-16-Ethoxy-9-hexadecen-1-amine	4942043	-8.0	-8.7	-5.0
(7E)-7-Hexadecen-1-amine	4942042	-5.1	-6.9	-8.0
N-[(3S)-3-(2-Methyl-2-propanyl)octyl]-1-octadecanamine	4678088	-5.3	-8.0	-9.8
MFCDD00010649	3397859	-5.0	-8.3	-8.5
[1,2,4]Triazolo[1,5-a]pyrimidin-2-amine	2412809	-6.1	-8.0	-8.7
1-Henicosanamine	2342457	-5.6	-5.0	-5.1
8,8-Dimethyl-1-nonanamine	2298922	-6.9	-6.5	-6.0
N, N – bis (14-ethoxytetradecyl)- 1, 3 – Propane Di Amine	2342349	-6.0	-6.0	-6.2

N, N – bis (3- Amino propyl) -1,36-hexatriacontanediamine	2342348	-6.2	-6.2	-5.9
10-Ethoxy-9,9-dimethyl-1-decanamine	2342304	-5.9	-5.9	-5.8
N,N-Bis(3-ethoxypropyl)-1-tetradecanamine	2342300	-5.8	-5.8	-6.0
N,N-Bis(3-ethoxypropyl)-1-octanamine	2342299	-6.0	-6.0	-4.9
[(4,6-Diamino-1,3,5-triazin-2-yl)(methoxymethyl)amino]methyl stearate	2285502	-4.9	-4.9	-5.0
DAS-Na	2285439	-5.0	-5.0	-6.2
(Z)-2-Hexene	558930	-6.1	-6.2	-5.8
BCE	143245	-5.8	-5.8	-5.3
Cholesterol Formate	144840	-8.1	-8.5	-5.2
tetra-O-(hydroxyethyl)rutoside	145097	-8.0	-8.7	-5.0
1-Isopropyl-6,9-dimethyl-5-oxatetracyclo[5.4.0.0 <sup>2,9</sup> .	381205	-4.9	-4.9	-5.0

0 <sup>4,6</sup> ]undecane - 3,3,6',9'- tetramethylspiro[oxiran e-2,2'- tricyclo[4.4.0.0 <sup>3,9</sup> ]decan e] (1:1)				
MFCD00005056	546318	-5.0	-5.0	-6.2
Hex-3-ene	553629	-6.1	-6.2	-5.8
(2E)-2-Hexene	555073	-5.8	-5.8	-5.3
Z-3-hexene	558881	-8.1	-8.5	-5.2
<i>Cassia fistula</i>				
Hex-2-ulosonic acid	49	-4.1	-4.7	-5.1
Hex-5-ulosonic acid	152	-4.9	-4.8	-4.0
6-O-Phosphonohexonic acid	409	-5.2	-4.1	-4.2
6-O-Phosphonohex-2- ulose	583	-5.3	-4.8	-4.5
Hexaric acid	587	-5	-4.8	-4.7
Hexopyranuronic acid	590	-5	-4.9	-4.0
4,5,6,7-Tetrahydroxy-2- oxo-8- (phosphonooxy)octanoi c acid	795	-5.6	-5.7	-5.5
2,3,4,5- Tetrahydroxypentanal	831	-4.8	-4.7	-4.5

1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid	1035	-5.6	-5.1	-5.7
7,8-Dimethyl-10-(2,3,4,5-Tetrahydroxypentyl)Benzoguanidinedione	1043	-7.1	-6.0	-6.1
2,3,4,5-Tetrahydroxy-6-oxo-1,7-heptanediyldibis(dihydrogen phosphate)	1156	-5.7	-5.6	-5.9
Cyanidanol	1166	-8.2	-7.9	-7.0
Vanillyl mandelic acid	1207	-6	-5.0	-5.3
3-Acetyl-3,12-dihydroxy-10-methoxy-6,11-Dioxo-1,2,3,4,6,11-Hexahydro-1-Tetracyclic-3-amino-2,3,6-Tri-Deoxy Hexopyranoside	1367	-8.7	-7.9	-8.0
5-thiohexose	1775	-4.6	-4.0	-4.1
(3,5-Dibromo-1,6-dihydroxy-4-methoxy-2,4-cyclohexadien-1-yl)acetonitrile	1957	-5.1	5.3	-5.0
Vakerin	2266	-6.4	-6.0	-5.9
Dimboa	2268	-6.7	-6.2	-6.0

Bisindolylmaleimide	2306	-8.6	-8.2	-8.5
2',4',3,4-Tetrahydroxy chalcone	2389	-8.0	-7.9	-7.3
2,16,20,25-tetra hydroxyl - 9, 10, 14 -Tri methyl - 4, 9 - Cyclo-9, 10- Seco cholesta -2, 5, 23 - Triene - 1, 11, 22 - Trione	2785	-7.5	-7.3	-7.2
5,6,10,10b- Tetrahydroxy- 3,4a,7,7,10a- pentamethyl-3- vinyl-dodecahydro-1H- benzo[f]chromen-1-one	2858	-7.1	-6.9	-7.2
5-(6-aminopurin-9-yl)- 2-methyl- tetrahydrofuran-3-ol	2936	-6.7	-5.9	-6.3
2- [amino (hydroxy) methylene] - 4 -(Di methyl amino) -5, 10, 11, 12 a - Tetra hydroxyl - 6 - methyl - 4a, 5a, 6, 12 a- tetrahydro-1, 3, 12 (2H, 4H, 5H) - tetra cetrione	3049	-8	-6.5	-7.9
12- fluoro - 6b - Glycoloyl -5- Hydroxy- 4a, 6a, 8, 8- Tetra	3262	-6.5	-6.2	-6.6

<p>methyl-4a, 4b, 5, 6, 6a, 6b, 9a, 10, 10a, 10b, 11, 12- Dodecahydro-2h-naphtho [2, 1: 4, 5] Indeno [1, 2 -d] [1, 3] Di oxol- 2- One</p>				
<p>4B, 12 – di fluoro - 6b – glycoloyl -5- hydroxy- 4a, 6a, 8, 8- tetra methyl-4a, 4b, 5, 6, 6a, 6b, 9a, 10, 10a, 10b, 11, 12 – Dodecahydro – 2h-Naphtho [2', 1' : 4, 5] Indeno [1, 2 -d] [1, 3] Di oxol – 2 - One</p>	3264	-7.0	-6.7	-6.9
<p>2-(4B, 12 – di fluoro -5- Hydroxy - 4 a, 6 a, 8, 8- Tetra methyl -2- Oxo-2, 4a, 4b, 5, 6, 6a, 9a, 10, 10a, 10b, 11, 12 - Dodeca hydro- 6b h- naphtho [2, 1:4, 5] Indeno [1, 2 -d] [1, 3] di oxol- 6b- yl) -2-Oxo Ethyl Acetate</p>	3265	-7.1	-6.8	-7.2
<p>12-fluoro - 6b – Glycoloyl -5- Hydroxy - 4A, 6A, 8, 8 – Tetra methyl-3, 4, 4a, 4b, 5, 6, 6A, 6B, 9A, 10, 10A, 10B, 11, 12-Tetra Deca hydro-2h-naphtho [2',</p>	3275	-6.6	-6.1	-6.3

1': 4, 5] indeno [1, 2 - d] [1, 3] Di Oxol -2- One				
Iopanoic acid	3604	-5.0	-4.9	-5.1
2-({3-Acetamido-5- [acetyl(methyl)amino]- 2,4,6- triiodobenzoyl} amino)- 2-deoxyhexose	4028	-5.9	-5.6	-5.5
Phenylbutazone	4617	-6.3	-6.0	-6.1
9A- acetoxy – 4A, 7B- Di hydroxyl - 3 - (Hydroxy methyl) - 1,1,6,8-tetramethyl-5- oxo- 1a,1b,4,4a,5,7a,7b,8,9,9 a-decahydro-1H- cyclopropa[3,4]benzo[1, 2-e]azulen-9-yl myristate	4628	-6.7	-6.5	-6.6
6,7-Diacetoxy- 4,14,16,20-Tetra hydroxyl -15- [(2- methyl butanoyl) oxy] - 4, 9 – Epoxy cevan -3- yl 2-Hydroxy -2-Methyl butanoate	4803	-7.2	-7.0	-6.8
6, 7- di acetoxy-4, 14, 16, 20-Tetra hydroxy- 15- [(2-methyl butanoyl) oxy]-4,9-	4804	-6.4	-6.1	-6.0



epoxy cevan-3-yl 2,3-Di hydroxy-2-Methyl butanoate				
Quinalizarin	4829	-8	-7.9	-7.2
2-Deoxy-2- {[methyl(nitroso)carba moyl]amino}hexose	5108	-5.6	-5.8	-5.5
Tetroquinone	5231	-5.3	-5.0	-5.1
[2,6-Dihydroxy-4- methoxy-3,5-bis(3- methyl-2-buten-1- yl)phenyl](4- hydroxyphenyl)methano ne	5473	-7.8	-6.9	-6.8
Tetrahydrocortisol	5655	-8.7	-8.3	-8.4
Fluocinolone Acetonide	5980	-6.9	-6.7	-6.5
Triamcinolone diacetate	5981	-7.2	-7.0	-7.1
Triamcinolone acetonide	6196	-6.7	-5.9	-5.2
Dihydroxytartaric Acid	6199	-6.1	-6.0	-6.2
(3R,5R)-1,3,4,5- Tetrahydroxycyclohexa necarboxylic acid	6262	-5.5	5.3	-5.0
1,4,5,8- Tetrahydroxyanthraquin one	6432	-8	-7.0	-6.9
Xanthone	6753	-8.2	-8.0	-7.8

Lactobionic acid	7040	-5.4	-5.2	-5.5
Desaspidin	7943	-6.8	-6.7	-6.3
Dioxybenzone	8251	-8	-7.3	-7.0
DJ1892000	8253	-7.7	-6.8	-7.2
D-(+)-Catechin	8711	-8.4	-8.5	-8.3
Fluocinonide	9265	-6.9	-6.5	-5.9
Hematein	9732	-8.2	-6.2	-7.6
5,8- di hydroxyl – 2 – Methoxy – 6 – Methyl - 7- (2-Oxopropyl)-1, 4- naphthoquinone	9743	-7.3	-6.8	-6.9
Catenarin	9744	-8.3	-7.8	-7.3
Cotoin	9768	-7.3	-7.0	-7.1
Hesperidin	10176	-8.4	-7.2	-8.1
Parietin	10193	-8.4	-7.7	-8.3
Flavone	10230	-8.4	-7.7	-7.5
(3 $\beta$ ,5 $\beta$ ,8 $\xi$ ,9 $\xi$ )-3,5,14,19- Tetrahydroxycard- 20(22)-enolide	10746	-8.9	-7.8	-8.2
1,7,8,9-Tetrahydroxy- 6H-benzo[7]annulen-6- one	10822	-7.2	-6.7	-7.0
Melibiose	10974	-6.2	-6.0	-5.8
(1 $\beta$ eta, 3 $\beta$ eta, 5 $\beta$ eta, 8 $\xi$ , 9 $\xi$ , 11 $\alpha$ eta) -3- [(6-de	11913	-6.6	-6.5	-6.2

oxy- $\alpha$ -L -Manno pyranosyl) oxy]-1, 5, 11, 14, 19-Penta hydroxyl card -20 (22)- enolide				
7-hydroxyemodin	12030	-8.4	-7.8	-7.9
6-Methoxy-tetralone	13490	-7.6	-7.7	-7.4
Carmine	14068	-8	-8.0	-7.8
1- [3- (3-butyryl-2, 4- Di hydroxy- 6-Methoxy benzyl) - 2, 6-Di hydroxyl - 4 - Methoxy -5- Methyl phenyl]- 1- Butanone	14456	-6.3	-6.2	-6.5
Fludrocortide	14475	-6.9	-6.7	-6.4
1-[3-(3-Butyryl-2,4- dihydroxy-6- methoxybenzyl)-2,4,6- trihydroxy-5- methylphenyl]-1- butanone	14903	-6.5	-6.3	-6.4
1-[3- (3- butyryl -2, 4- Di hydroxy-6-Methoxy- 5-Methyl Benzyl) -2, 4, 6- tri hydroxyl -5- methyl phenyl] -1- Butanone	15068	-6.6	-6.7	-6.2

Octachloronaphthalene	15827	-6.1	-6.5	-6.3
2-[(4bR,6bS,9aR)-2-(2-chloro ethoxy) -4 B-Fluoro -12- Formyl -5-Hydroxy – 4A, 6A, 8, 8-Tetra methyl-3, 4, 4A, 4B, 5, 6, 6A, 9A, 10, 10 A,10 B, 11-Dodeca hydro – 6b h-Naphtho [2', 1':4, 5] Indeno [1,2-D] [1, 3] Di oxol- 6B-yl] -2- Oxo Ethyl Acetate	16814	-6.4	-6.0	-5.7
Tetrahydropapaveroline hydrochloride	17490	-7.8	-7.0	-6.6
Iduronic acid	17794	-5.5	-4.8	-4.9
TTP	17927	-6.6	-6.5	-6.3
Physson-9-anthrone	18052	-8.9	-7.7	-6.9
3-O-methylgallic acid	18679	-6.0	-6.4	-6.1
MFCDD01673157	18680	-5.9	-6.0	-5.6
MFCDD00053302	19202	-4.0	-3.9	-4.1
5,8-dihydroxy-7-methoxy-2-phenylchromen-4-one	19296	-4.8	-4.0	-4.2
Triamcinolone hexacetonide	20516	-4.2	-4.5	-4.3
Dermoglucine	22083	-4.1	-4.5	-3.3
Norsolorinic acid	23449	-4.8	-6.0	-5.7

Calcium glubionate monohydrate	23833	-4.1	-4.0	-3.6
Tetra Meta Phosphoric Acid	24178	-4.6	-4.5	-3.9
12-O-Tetradecanoylphorbol-13-acetate	25977	-5.0	-5.4	-6.0
(1s)-5-deoxy-1-c-[(2s,3s)-7-[[2,6-Dideoxy-3-o-(2,6-Dideoxy-beta-D-Arabinohexopyranosyl)-beta-D-Arabinohexopyranosyl]oxy]-3-[[2,6-Dideoxy-3-C-Methyl-beta-D-Ribohexopyranosyl-(1->3)-2,6-Dideoxy-beta-D-Arabinohexopyranosyl-(1->3)-2,6-Dideoxy-beta-D-Arabinohexopyranosyl]oxy]-5,10-Dihydroxy-6-Methyl-4-Oxo-1,2,3,4-Tetrahydro-2-Anthracenyl]-1-O-Methyl-D-Xylulose	27026	-5.2	-5.7	-5.9
1,2,3,4-tetrahydro-1,3,4,5,10-pentahydroxyl-2-methyl-3-	27176	-5.4	-6.3	-6.0

[(3-Methyl oxo ranyl) carbonyl]-4A, 9A-Epoxy Anthracen-9 (10 H) – One				
3A, 10, 11, 11A-tetra hydroxyl - 9, 15 A- di methyl-1- (5-Oxo- 2, 5- Di hydro -3-furanyl) Icosa hydro -7Ah, 13Ah-cyclopenta [7,8] Phenanthro [2,3-B] Pyrano [3, 2-e] [1,4] Dioxine -13A- Carbaldehyde	27960	-5.6	-6.1	-5.8
Pedalin	28911	-5.4	-3.9	-4.1
Triamcinolone	29046	-5.0	-4.8	-4.9
Nivalenol	29515	-4.9	-4.8	-4.5
(3 $\alpha$ ,4 $\alpha$ ,5 $\xi$ ,8 $\xi$ ,9 $\beta$ ,13 $\xi$ ,16 $\beta$ )-12,14,17,20-Tetrahydroxy-4,9-epoxycevan-3,4,16-triyl triacetate	29900	-4.2	-4.0	-4.1
2-(3,4-Diacetoxyphenyl)-7-methoxy-4-oxo-4H-chromene-5,6-diyl diacetate	30653	-4.8	-4.6	-4.7
(1 $\beta$ , 5 $\xi$ , 6 $\alpha$ , 9 $\xi$ , 13 $\alpha$ ,14r)-1, 6, 7, 14-tetra hydroxy-7, 20-	31656	-5.0	-4.9	-4.8

Epoxy kaur-16-en-15-One				
2-(3,4-Dihydroxy-5-methoxybenzoyl)hydrazinecarboximidamide	34133	-5.2	-5.1	-5.0
(3S,5S)-1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid	34345	-5.1	-5.0	-4.9

#### 4.2 Computational study of plants against *Cx. quinquefasciatus*

The mol files of the ligands and three dimensional structures of the secondary metabolites from *Cx. quinquefasciatus* were mentioned as an input in the CB-dock server for blind docking. The CB-dock server provides all information regarding the activity of secondary metabolites. The activities of the secondary metabolites and compounds shown in the Table 4.2. In the present study, phytoconstituents of different plants have revealed a significant level of docking score and interaction energies against *Cx. quinquefasciatus* proteins 2C9K, 5W1U and 2WLS. The highest interaction energy score was found to be  $-11.2$  Kcal/mol against 2WLS protein of *Cx. quinquefasciatus*. The highest interaction energy score was found by the phytoconstitute of *C. procera* followed by *A. americana* and *A. marmelos* respectively.

It has been suggested that the acetylcholinesterase (AChE) enzyme can be inhibited, which would have a similar neurotoxicity effect to that of organophosphorus as well as carbamate insecticides (Isman, 2000). Eugenol with  $\alpha$ -terpineol have been shown to possess similar effects on flies as well as cockroaches (Houghton *et al.*, 2006). However, several writers concur that there is typically no connection between AChE inhibition as well as the larvicidal properties of terpenes and their derivatives. Additionally, according to Kumar *et al.*, (2012), terpenes found in *Calotropis gigantea* possess larvicidal effect because they can obstruct the sterol transport protein, and that is partly in charge of transporting cholesterol intracellularly in insects (Priestley *et al.*, 2003). SCP-2 is present in high levels in the larvae throughout the feeding phase because they are dependent on external supplies of cholesterol for the manufacture of steroid derivatives (Kumar *et al.*, 2012). The potential for drugs that can block this protein as vector control agents is therefore very considerable.

**Table 4.2:** Tabular display of docking score of the *Culex* larval essential proteins with phyto constituents

<b>Name of Phytoconstitute</b>	<b>ChemSpider ID of Phyto constituents</b>	<b>Interaction energy (kcal/mol) against 2C9K protein</b>	<b>Interaction energy (kcal/mol) against 5W1U protein</b>	<b>Interaction energy (kcal/mol) against 2WLS protein</b>
<b><i>Cannabis sativa</i></b>				
$\gamma$ -Terpinene	7181	-5.5	-6.3	-6.5
$\beta$ -Terpinene	60205	-5.4	-6.4	-6.6
Linalool	6402	-5.1	-6.5	-6.6
Linalool	391430	-5.2	-6.0	-6.0
trans-Anethole	553166	-5.4	-6.5	-6.7
Linalool	1266019	-6.4	-6.7	-6.8
Linalool	13849981	-5.3	-6.0	-6.1
<b><i>Calotropis procera</i></b>				
Urs-19(29)-en-3-yl acetate	164675	-9.4	-9.4	-9.5
Sitosterol	192962	-7.9	-9.0	-11.0
Stigmasterol	4444352	-7.9	-8.9	-11.2
Multiflorenol	32700975	-8.7	-10.2	-10.0
In00242	64870692	-9.1	-10.0	9.9
<b><i>Datura stramonium</i></b>				
2-Chloro-4-Aminotoluene-5-Sulfonic Acid	6669	-6.2	-7.1	7.0
Acetoacet-o-chloranilide	6889	-6.2	-6.1	-6.3
2-Chloro-4-aminotoluene	6985	-6.1	-7.0	-7.1
Methyldiphenylamine	10627	-6.6	-7.2	-7.2
N,4-Dimethylaniline	11665	-5.1	-6.8	-7.1
Diazepam Related Compound A	13323	-7.3	-6.8	-7.0
3-Methyldiphenylamine	13910	-7.0	-7.3	-7.3



4-bromo-m-toluidine	21844	-6.1	-7.6	-6.3
$\alpha$ -Solanine	28033	-9.1	-8.2	-6.1
2-Ethyl-6-methylaniline	30109	-5.7	-6.8	-6.1
<i>Aegle marmelos</i>				
Ethyl (3,4-dichlorophenyl) acetate	149921	-6.0	-5.3	-5.3
Ethyl {2-[(4-methylphenyl)amino]-1,3-thiazol-4-yl} acetate	3953401	-7.3	-7.6	-8.3
Ethyl chloro((4-nitrophenyl)hydrazono)acetate	4736864	-7.4	-8.2	-8.1
Ethyl (2E)-chloro[(2-methylphenyl) hydrazono] acetate	4838953	-6.8	-6.8	-8.1
Ethyl (2E)-chloro[(2-fluorophenyl)hydrazono]acetate	4838956	-6.8	-6.8	-8.0
Ethyl (2E)-amino[(2,4-dichlorophenyl)hydrazono] acetate	9812399	-7.6	-7.3	-7.2
Ethyl (3,4-difluorophenyl)(difluoro)acetate	10325010	-6.7	-7.6	-7.2
Ethyl (4-chlorophenyl)(difluoro)acetate	14010415	-6.2	-7.1	-7.3
Ethyl difluoro(3-fluoro-4-methoxyphenyl)acetate	21391591	-6.8	-6.7	-6.8
Ethyl difluoro(3-methoxyphenyl)acetate	21391594	-6.3	-7.1	-7.4
Ethyl difluoro(4-isopropylphenyl)acetate	21391615	-6.9	-7.9	-8.2
Imperatorin	9797	-8.3	-8.4	-9.1
<i>Agave americana</i>				
(2S,3S,4S,5R,6R)-6-	52082957	-7.9	-8.3	-7.6

{[(2S,3S,4S,5R)-2- ({[(2R,3S,4S,5R)-3,4- Dihydroxy-2,5- bis(hydroxymethyl)tetrahydro- 2-furanyl]oxy}methyl)-3,4- dihydroxy-5- (hydroxymethyl)tetrahydro-2- furanyl]oxy} tetrahydro-2H- pyran-2 ,3,4,5-tetrol				
Isoflavone	65255	-7.9	-8.2	-9.7
Spirostan-3-yl 2-O-[2- (hexopyranosyloxy)-3,4,5,6 tetrahydrocyclohexyl]-4-O- pentopyranosylhexopyranoside	153288	-9.9	-11.0	-9.7
1-O-β-D-Fructofuranosyl-β-D- fructofuranose	388643	-7.6	-7.4	-7.3
1-O-β-D-Fructofuranosyl-β-D- fructofuranose	9182610	-10.1	-10.4	-10.0
(2S,3S,4S,5R,6R)-6- {[(2S,3S,4S,5R)-2- ({[(2R,3S,4S,5R)-3,4- Dihydroxy-2,5- bis(hydroxymethyl)tetrahydro- 2-furanyl]oxy}methyl)-3,4- dihydroxy-5- (hydroxymethyl)tetrahydro-2- furanyl]oxy} tetrahydro-2H- pyran-2 ,3,4,5-tetrol	52082957	-7.9	-8.3	-7.6
<b><i>Achyranthus aspera</i></b>				
12-O-Tetradecanoylphorbol-13- acetate Dihexadecyl ketone	25977	-6.7	-6.0	-6.1
16-Tritriacontanone - 8-	213083	-5.1	-5.6	-5.2

pentatriacontanone				
16-Tritriacontanone	213085	-5.0	-5.3	-6.0
Aconitic Acid	392201	-6.2	-5.1	-5.2
3-Tritriacontanone	474943	-6.1	-6.1	-6.6
2-Tritriacontanone	476843	-6.2	-6.0	-6.1
24,25-Bis(methylsulfanyl)-2-tritriacontanone	550318	-6.3	-6.3	-5.2
MFCD00026601	4366538	-5.6	-7.3	-5.3
12-O-Tetradecanoylphorbol-13-acetate	8651284	-5.2	-5.1	-5.4
$\alpha$ -D-Galp-(1->3)- $\beta$ -D-Galf-(1->3)- $\alpha$ -D-Manp-(1->3)- $\alpha$ -D-Manp-(1->4)- $\alpha$ -D-GlcpN-(1->6)-1D-myo-inositol	26332395	-5.8	-5.3	-5.1
<b><i>Eichhornia crassipes</i></b>				
Shikimic acid	9161960	-6.8	-8.0	-6.4
1,1-Diphenyl-2-(2,4,6-trinitrophenyl)diazenium	15122	-6.4	-5.6	-5.3
DPPH	66953	-5.9	-5.9	-4.5
Sitosterol	192962	-6.4	-5.9	-6.9
DPPH	2016757	-5.9	-5.9	-5.5
Stigmasterol	4444352	-6.1	-5.9	-5.2
<b><i>Mentha piperita</i></b>				
Piperitenone Oxide	390924	-5.9	-6.9	-5.6
Ot0175110	10756	-5.5	-6.4	-6.4
Piperitenone Oxide	55800	-6.3	-6.5	-5.4
<b><i>Catharanthus roseus</i></b>				
Urs-12-en-3-yl acetate	259299	-5.4	-6.1	-5.7
Oleanolic acid	10062	-5.1	-5.4	-4.6
$\beta$ -Amyrin acetate	83201	-4.2	-5.3	-5.4
266N1630AL	83811	-5.5	-5.6	-6.2
<b><i>Allium ampeloprasum</i></b>				
Limonene	389747	-5.4	-6.3	-6.1

Pinene	389794	-5.1	-6.2	-6.1
$\alpha$ -Pinene	389795	-5.2	-6.1	-5.7
$\alpha$ -Farnesene	4444849	-5.7	-5.4	-4.6
$\beta$ -Farnesene	4444850	-5.2	-5.3	-5.4
$\beta$ -Pinene	8466294	-5.1	-5.6	-6.2
Terpineol	13850142	-5.4	-6.3	-6.1
$\beta$ -D-Glucopyranoside, 2,4-dihydroxy-6-(2-(4-hydroxyphenyl)ethenyl)phenyl	19026512	-5.8	-5.2	-4.1
Ethane-d5	21170395	-2	-1.9	-1.0
Carbon tetrachloride	5730	-3	-2.0	-2.5
<i>Aloe vera</i>				
Salicylic acid	331	-5.9	-5.9	-5.5
Disodium (1Z)-N-[(7Z)-8-oxo-7-(phenylhydrazono)-6-sulfo-4-sulfonato-7,8-dihydro-1-naphthalenyl]ethanimidate	59696683	-6.1	-5.9	-5.2
2-Hydroxybenzoic acid - 2-(1-piperazinyl)ethanamine (1:1)	57461842	-5.9	-6.9	-5.6
2-Hydroxybenzoic acid - 1-butyl-1H-imidazole (1:1)	21165422	-5.5	-6.4	-6.4
(1 $\beta$ ,3 $\beta$ ,25R)-3-Hydroxyspirost-5-en-1-yl $\beta$ -D-glucopyranosyl-(1->2)-[ $\beta$ -D-xylopyranosyl-(1->3)]-6-deoxy- $\beta$ -D-galactopyranoside	9182610	-6.3	-6.5	-5.4
(1R,2S)-Ethyl 1-(Boc-amino)-2-vinylcyclopropanecarboxylate	8834028	-5.4	-6.1	-5.7
Imidazole salicylate	34333	-5.1	-5.4	-4.6
Caffeine salicylate	57699	-4.2	-5.3	-5.4
2-Hydroxybenzoic acid - 8-	58210	-5.5	-5.6	-6.2

methyl-8-azabicyclo[3.2.1]oct-3-yl 3-hydroxy-2-phenylpropanoate (1:1)				
Antipyrine Salicylate	91924	-5.4	-6.3	-6.1
<b><i>Lantana camara</i></b>				
Oleanonic acid	10194990	-5.4	-6.2	-6.1
DPPH	2016757	-8.8	-8.0	-7.9
(E)-Aconitic Acid	392201	-5.8	-5.7	-5.5
DPPH	66953	-7.9	-6.7	-7.7
1,1-Diphenyl-2-(2,4,6-trinitrophenyl)diazonium	15122	-8.4	-8.0	-7.9
<b><i>Ficus bengalensis</i></b>				
(25E)-25-Tetratriaconten-2-one	4517307	-5.2	-5.0	-5.1
Inositol	10239179	-6.3	-6.1	-6.0
(1R,2s,3S,4r)-1,2,3,4,5,6-Cyclohexanehexol	32696449	-5.8	-5.9	-4.0
<b><i>Eucalyptus camaldulensis</i></b>				
$\beta$ -Terpinene	60205	-5.4	-5.3	-5.0
(1S,5S)-4-Isopropyl-1-methyl-6-oxabicyclo[3.1.1]heptanes	59696307	-5.4	-4.7	-5.7
(1R,6R)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-ol	58837155	-5.7	-6.0	-7.0
(1R,6S)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-ol	58829897	-5.5	-5.0	-5.1
1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-ol - acetic acid (1:1)	57465927	-7.1	-5.1	-6.2
1,3,3-Trimethyl-2-	57257156	-5.5	-5.1	-4.5

oxabicyclo[2.2.2]octane				
(4R,6S)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-ol	57256935	-5.7	-5.2	-5.1
(1r,4r)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane	31045960	-5.5	-6.0	-6.7
(1S,4R,6S)-3,3-Dimethyl-2-oxabicyclo[2.2.2]octan-6-ol	30783268	-5.4	-6.0	-6.9
OS9274000	28295867	-5.4	-5.1	-5.3
<b><i>Ocimum sanctum</i></b>				
Methylamine	6089	-2.2	-3.5	-4.0
(1S,3R,5R,6R,8R,10R,11R,13R,15R,16R,18R,20R,21R,23R,25R,26R,28R,30R,31S,33R,35R,36R,37S,40R,41S,42R,43S,44R,45S,46R,47S,48R,49S)-5,10,15,20,25,30,35-Heptakis(hydroxymethyl)-37,39,40,41,42,43,44,45,46,47,48,49-dodecamethoxy-2,4,7,9,12,14,17,19,22,24,27,29,32,34-tetradecaoxaoctacyclo[31.2.2.2 <sup>3,6</sup> .2 <sup>8,11</sup> .2 <sup>13,16</sup> .2 <sup>18,21</sup> .2 <sup>23,26</sup> .2 <sup>28,31</sup> ]nonatetracontane-36,38-diol	64849692	-6.6	-6.0	-6.0
(1S,3R,5R,6R,8R,10R,11R,13R,15R,16R,18R,20R,21R,23S,25R,26R,28R,30R,31S,33R,35R,36R,37S,38R,39S,40R,41S,42R,43S,44R,45S,46R,47S,48R,49S)-5,10,15,20,25,30,35-	57620332	-5.7	-5.3	-5.1

Heptakis(hydroxymethyl)- 37,39,40,41,42,43,4 4,45,46,47,48,49- dodecamethoxy- 2,4,7,9,12,14,17,19,22,24,27,29,3 2,34- tetradecaoxaocyclo[31.2.2.2 <sup>3,6</sup> . 2 <sup>8,11</sup> .2 <sup>13,16</sup> .2 <sup>18,21</sup> .2 <sup>23,26</sup> .2 <sup>28,31</sup> ]nonat etracontane-36,38-diol				
(4R,5S,6S,7S,4'R,5'S,6'S,7'S)- 2,2'- [Oxybis(methylene)]bis(3,4,5,6,7 ,8-hexahydroxyoctanenitrile)	57488057	-8.0	-8.1	-7.7
2-O-(2-Hydroxyethyl)-4-O-[2-O- (2- hydroxypropyl)hexopyranosyl]h exopyranose	57487300	-8.9	-8.0	-7.9
3-(Allyloxy)-1-propene - $\alpha$ -D- fructofuranosyl $\alpha$ -D- glucopyranoside (1:1)	57486960	-9.7	-9.5	-5.1
2,4,5-T Dimethylamine	21418	-7.6	-6.6	-6.1
2,4,5-T-trolamine	18522	-9.3	-8.9	-7.0
MFCD00027307	17558	-4.4	-5.1	-5.5
2,4,5-T-Triethylammonium	15358	-8.0	-6.7	-7.9

### 4.3 Computational study of plants against *An. stephensi*

The mol files of the ligands and three dimensional structures of the secondary metabolites from *An. stephensi* were mentioned as an input in CB-dock server for blind docking. The CB-dock server provides all information regarding activity of secondary metabolites. The activities of the secondary metabolites and compounds shown in Table 4.3. In the present study, phyto constituents of different plants have revealed the significant level of docking score and interaction energies against *An. stephensi* proteins 3NGV, 3NHT and 4OKV. The highest interaction energy score was found to be  $-9.8$  Kcal/mol against 3NHT protein of *An. stephensi*. The highest interaction energy score was found by the phytoconstitute of *C. procera* followed by *A. marmelos* and *A. americana* respectively.

Similar results were shown by Devi *et al.*, (2010), in which Inter - molecular flexibility docking simulation of the kappa-carrageenan interactions with the D7 proteins were run, and energy levels were determined from the docked morphologies of the inhibitor protein complexes. Important knowledge on the positioning of the inhibitors in the target protein's binding pocket was obtained via docking experiments. The docking simulation has helped to identify a number of possible inhibitors. Kcal/mol was used to calculate the D7 protein's affinity for the kappa-carrageenan. The greater interaction was determined to have a docking score of  $-8.67$  Kcal/mol. The anti-repellant efficacy towards the *An. stephensi* mosquito can benefit from an investigation of the ligand bind association with the protein. The findings of this study will help us comprehend how the inhibitory mode works as well as quickly and precisely forecast how larvicidal will function based on molecular docking. The *An. stephensi* 3NHT protein was determined to have the greatest interaction energy score, which was  $-9.8$  Kcal/mol. The phytoconstituents of *C. procera* received the highest interaction energy rating, followed by *A. marmelos* and *A. americana*, in that order.



**Table 4.3:** Tabular display of docking score of the *Anopheles* larval essential proteins with phyto constituents

Name of Phytoconstitute	ChemSpider ID of Phyto constituents	Interaction energy (kcal/mol) against 3NGV protein	Interaction energy (kcal/mol) against 3NHT protein	Interaction energy (kcal/mol) against 4OKV protein
<b><i>Cannabis sativa</i></b>				
$\gamma$ -Terpinene	7181	2.2	3.1	3.0
$\beta$ -Terpinene	60205	3.0	3.5	3.2
Linalool	6402	3.8	3.5	3.3
Linalool	391430	4.0	3.9	3.5
trans-Anethole	553166	3.1	3.2	3.3
Linalool	1266019	3.5	3.4	3.8
Linalool	13849981	3.6	3.7	3.5
<b><i>Calotropis procera</i></b>				
Urs-19(29)-en-3-yl acetate	164675	-8.7	-9.2	-8.8
Sitosterol	192962	-7.8	8.3	-7.7
Stigmasterol	4444352	-8.1	-8.2	-7.8
Multiflorenol	32700975	-9.4	-9.0	-9.2
In00242	64870692	-9.1	-9.8	-9.3
<b><i>Datura stramonium</i></b>				
2-Chloro-4-Aminotoluene-5-Sulfonic Acid	6669	6.0	6.1	5.9
Acetoacet-o-chloranilide	6889	6.2	6.3	6.0
2-Chloro-4-aminotoluene	6985	6.1	6.2	6.0
Methyldiphenylamine	10627	5.9	5.6	6.1
N,4-Dimethylaniline	11665	6.3	6.0	5.7
Diazepam Related Compound A	13323	6.5	6.2	5.5

3-Methyldiphenylamine	13910	6.1	6.0	6.3
4-bromo-m-toluidine	21844	6.4	6.2	6.1
$\alpha$ -Solanine	28033	6.4	5.9	6.0
2-Ethyl-6-methylaniline	30109	6.0	6.3	5.9
<i>Aegle marmelos</i>				
Ethyl (3,4-dichlorophenyl)acetate	149921	-6.6	-6.5	-6.6
Ethyl {2-[(4-methylphenyl)amino]-1,3-thiazol-4-yl}acetate	3953401	-8.4	-8.4	-7.8
Ethyl chloro((4-nitrophenyl)hydrazono)acetate	4736864	-6.0	-8.5	-7.3
Ethyl (2E)-chloro[(2-methylphenyl)hydrazono]acetate	4838953	-6.6	-6.3	-6.5
Ethyl (2E)-chloro[(2-fluorophenyl)hydrazono]acetate	4838956	-6.8	-7.8	-6.7
Ethyl (2E)-amino[(2,4-dichlorophenyl)hydrazono]acetate	9812399	-7.2	-7.1	-7.1
Ethyl (3,4-difluorophenyl)(difluoro)acetate	10325010	-7.0	-6.7	-7.2
Ethyl (4-chlorophenyl)(difluoro)acetate	14010415	-7.1	-7.0	-7.1
Ethyl difluoro(3-fluoro-4-methoxyphenyl)acetate	21391591	-7.3	-7.2	-7.1
Ethyl difluoro(3-methoxyphenyl)acetate	21391594	-6.9	-7.9	-6.9
Ethyl difluoro(4-isopropylphenyl)acetate	21391615	-7.0	-7.3	-7.3
Imperatorin	9797	-8.0	-7.9	-8.1
<i>Agave americana</i>				

(2S,3S,4S,5R,6R)-6- {[(2S,3S,4S,5R)-2- ({(2R,3S,4S,5R)-3,4- Dihydroxy-2,5- bis(hydroxymethyl)tetrahydr o-2-furanyl]oxy}methyl)- 3,4-dihydroxy-5- (hydroxymethyl)tetrahydro- 2-furanyl]oxy}tetrahydro- 2H-pyran-2 ,3,4,5-tetrol	52082957	-6.2	-6.6	-6.7
Isoflavone	65255	-6.2	-6.8	-6.4
Spirostan-3-yl 2-O-[2- (hexopyranosyloxy)-3,4,5,6 tetrahydrocyclohexyl]-4- O- pentopyranosylhexopyranosi de	153288	-6.4	-6.5	-7.1
1-O-β-D-Fructofuranosyl-β- D-fructofuranose	388643	-7.0	-7.7	-6.2
1-O-β-D-Fructofuranosyl-β- D-fructofuranose	9182610	-7.1	-7.0	-7.1
(2S,3S,4S,5R,6R)-6- {[(2S,3S,4S,5R)-2- ({(2R,3S,4S,5R)-3,4- Dihydroxy-2,5- bis(hydroxymethyl)tetrahydr o-2-furanyl]oxy}methyl)- 3,4-dihydroxy-5- (hydroxymethyl)tetrahydro- 2-furanyl]oxy}tetrahydro- 2H-pyran-2 ,3,4,5-tetrol	52082957	-7.3	-7.2	-8.0
<b><i>Achyranthus aspera</i></b>				
12-O-Tetradecanoylphorbol- 13-acetate	25977	-6.0	-5.9	-6.0
Dihexadecyl ketone		-6.1	-6.3	-6.2
16-Tritriacontanone - 8- pentatriacontanone	213083	-5.9	-5.9	-4.5
16-Tritriacontanone	213085	-6.4	-5.9	-6.9
Aconitic Acid	392201	-5.9	-5.9	-5.5

3-Tritriacontanone	474943	-6.1	-5.9	-5.2
2-Tritriacontanone	476843	-5.9	-6.9	-5.6
24,25-Bis(methylsulfanyl)-2-tritriacontanone	550318	-5.5	-6.4	-6.4
MFCD00026601	4366538	-6.3	-6.5	-5.4
12-O-Tetradecanoylphorbol-13-acetate	8651284	-5.4	-6.1	-5.7
$\alpha$ -D-Galp-(1->3)- $\beta$ -D-Galf-(1->3)- $\alpha$ -D-Manp-(1->3)- $\alpha$ -D-Manp-(1->4)- $\alpha$ -D-GlcpN-(1->6)-1D-myo-inositol	26332395	-5.1	-5.4	-4.6
<b><i>Eichhornia crassipes</i></b>				
Shikimic acid	9161960	-4.2	-5.3	-5.4
1,1-Diphenyl-2-(2,4,6-trinitrophenyl)diazonium	15122	-5.4	-6.3	-6.1
DPPH	66953	-5.1	-6.2	-6.1
Sitosterol	192962	-5.2	-6.1	-5.7
DPPH	2016757	-5.7	-5.4	-4.6
Stigmasterol	4444352	-5.2	-5.3	-5.4
<b><i>Mentha piperita</i></b>				
Piperitenone Oxide	390924	-5.1	-5.6	-6.2
Ot0175110	10756	-5.4	-6.3	-6.1
Piperitenone Oxide	55800	-5.8	-5.2	-4.1
<b><i>Catharanthus roseus</i></b>				
Urs-12-en-3-yl acetate	259299	-2	-1.9	-1.0
Oleanolic acid	10062	-3	-2.0	-2.5
$\beta$ -Amyrin acetate	83201	-5.9	-5.9	-5.5
266N1630AL	83811	-6.1	-5.9	-5.2
<b><i>Allium ampeloprasum</i></b>				
Limonene	389747	-5.9	-6.9	-5.6
Pinene	389794	-5.5	-6.4	-6.4

$\alpha$ -Pinene	389795	-6.3	-6.5	-5.4
$\alpha$ -Farnesene	4444849	-5.4	-6.1	-5.7
$\beta$ -Farnesene	4444850	-5.1	-5.4	-4.6
$\beta$ -Pinene	8466294	-4.2	-5.3	-5.4
Terpineol	13850142	-5.5	-5.6	-6.2
$\beta$ -D-Glucopyranoside, 2,4-dihydroxy-6-(2-(4-hydroxyphenyl)ethenyl)phenyl	19026512	-5.4	-6.3	-6.1
Ethane-d5	21170395	-5.4	-6.2	-6.1
Carbon tetrachloride	5730	-5.9	-5.9	-4.5
<i>Aloe vera</i>				
Salicylic acid	331	-6.4	-5.9	-6.9
Disodium (1Z)-N-[(7Z)-8-oxo-7-(phenylhydrazono)-6-sulfo-4-sulfonato-7,8-dihydro-1-naphthalenyl]ethanimidate	59696683	-5.9	-5.9	-5.5
2-Hydroxybenzoic acid - 2-(1-piperazinyl)ethanamine (1:1)	57461842	-6.1	-5.9	-5.2
2-Hydroxybenzoic acid - 1-butyl-1H-imidazole (1:1)	21165422	-5.9	-6.9	-5.6
(1 $\beta$ ,3 $\beta$ ,25R)-3-Hydroxyspirost-5-en-1-yl $\beta$ -D-glucopyranosyl-(1->2)-[ $\beta$ -D-xylopyranosyl-(1->3)]-6-deoxy- $\beta$ -D-galactopyranoside	9182610	-5.5	-6.4	-6.4
(1R,2S)-Ethyl 1-(Boc-amino)-2-vinylcyclopropanecarboxylate	8834028	-6.3	-6.5	-5.4
Imidazole salicylate	34333	-5.4	-6.1	-5.7
Caffeine salicylate	57699	-5.1	-5.4	-4.6

2-Hydroxybenzoic acid - 8-methyl-8-azabicyclo[3.2.1]oct-3-yl 3-hydroxy-2-phenylpropanoate (1:1)	58210	-4.2	-5.3	-5.4
Antipyrine Salicylate	91924	-5.5	-5.6	-6.2
<b><i>Lantana camara</i></b>				
Oleanonic acid	10194990	-5.4	-6.3	-6.1
DPPH	2016757	-5.1	-6.2	-6.1
(E)-Aconitic Acid	392201	-5.2	-6.1	-5.7
DPPH	66953	-5.7	-5.4	-4.6
1,1-Diphenyl-2-(2,4,6-trinitrophenyl)diazonium	15122	-5.2	-5.3	-5.4
<b><i>Ficus bengalensis</i></b>				
(25E)-25-Tetratriaconten-2-one	4517307	-5.1	-5.6	-6.2
Inositol	10239179	-5.4	-6.3	-6.1
(1R,2s,3S,4r)-1,2,3,4,5,6-Cyclohexanehexol	32696449	-5.8	-5.2	-4.1
<b><i>Eucalyptus camaldulensis</i></b>				
$\beta$ -Terpinene	60205	-2	-1.9	-1.0
(1S,5S)-4-Isopropyl-1-methyl-6-oxabicyclo[3.1.1]heptanes	59696307	-3	-2.0	-2.5
(1R,6R)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-ol	58837155	-5.9	-5.9	-5.5
(1R,6S)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-ol	58829897	-6.1	-5.9	-5.2
1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-ol - acetic acid (1:1)	57465927	-5.9	-6.9	-5.6
1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane	57257156	-5.5	-6.4	-6.4

(4R,6S)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-ol	57256935	-6.3	-6.5	-5.4
(1r,4r)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane	31045960	-5.4	-6.1	-5.7
(1S,4R,6S)-3,3-Dimethyl-2-oxabicyclo[2.2.2]octan-6-ol	30783268	-5.1	-5.4	-4.6
OS9274000	28295867	-4.2	-5.3	-5.4
<i>Ocimum sanctum</i>				
Methylamine	6089	-5.5	-5.6	-6.2
(1S,3R,5R,6R,8R,10R,11R,13R,15R,16R,18R,20R,21R,23R,25R,26R,28R,30R,31S,33R,35R,36R,37S,40R,41S,42R,43S,44R,45S,46R,47S,48R,49S)-5,10,15,20,25,30,35-Heptakis(hydroxymethyl)-37,39,40,41,42,43,44,45,46,47,48,49-dodecamethoxy-2,4,7,9,12,14,17,19,22,24,27,29,32,34-tetradecaoxaoctacyclo[31.2.2.2.nonatetracontane-36,38-diol	64849692	-5.4	-6.3	-6.1
(1S,3R,5R,6R,8R,10R,11R,13R,15R,16R,18R,20R,21R,23S,25R,26R,28R,30R,31S,33R,35R,36R,37S,38R,39S,40R,41S,42R,43S,44R,45S,46R,47S,48R,49S)-5,10,15,20,25,30,35-Heptakis(hydroxymethyl)-37,39,40,41,42,43,44,45,46,47,48,49-dodecamethoxy-2,4,7,9,12,14,17,19,22,24,27,29,32,34-tetradecaoxaoctacyclononate tracontane-36,38-diol	57620332	-5.4	-6.2	-6.1
(4R,5S,6S,7S,4'R,5'S,6'S,7'S)-2,2'-[Oxybis(methylene)]bis(3,4,5,6,7,8-	57488057	-5.9	-5.9	-4.5

hexahydroxyoctanenitrile)				
2-O-(2-Hydroxyethyl)-4-O-[2-O-(2-hydroxypropyl)hexopyranosyl]hexopyranose	57487300	-6.4	-5.9	-6.9
3-(Allyloxy)-1-propene - $\alpha$ -D-fructofuranosyl $\alpha$ -D-glucopyranoside (1:1)	57486960	-5.9	-5.9	-5.5
2,4,5-T Dimethylamine	21418	-6.1	-5.9	-5.2
2,4,5-T-trolamine	18522	-5.9	-6.9	-5.6
MFCD00027307	17558	-5.5	-6.4	-6.4
2,4,5-T-Triethylammonium	15358	-6.3	-6.5	-5.4



#### 4.4. Larvicidal activity of *C. procera* leaves extract against *Ae. aegypti*.

Larvicidal activity of hexane, acetone, ethanol, and aqueous extract of *C. procera* tested against the 4<sup>th</sup> instar larvae of *Ae. aegypti* is represented below (Table 4.4 and Figure 4.1). It is pertinently noted that, larval mortality was increased with the concentration of extract. The strongest larvicidal activity was demonstrated by the ethanol extract of *C. procera* leaves.

The LC<sub>50</sub> value of ethanol extract was 1.923ppm and LC<sub>90</sub> value was 8.83ppm with regression equation  $Y=1.936X-0.549$ , 95% confidence limit LCL<sub>50</sub> of 1.56ppm, LCL<sub>90</sub> of 6.58ppm and UCL<sub>50</sub> of 2.33ppm and UCL<sub>90</sub> of 13.39ppm while the hexane extract showed the lowest larvicidal activity with LC<sub>50</sub> value 5.364ppm and LC<sub>90</sub> value was 31.759ppm with regression equation  $Y=1.659X-1.21$ , 95% confidence limit LCL<sub>50</sub> of 4.52ppm, LCL<sub>90</sub> of 24.35ppm and UCL<sub>50</sub> of 6.27ppm and UCL<sub>90</sub> of 45.30ppm. Aqueous extract (LC<sub>50</sub> value 2.607ppm and LC<sub>90</sub> value was 11.903ppm with regression equation  $Y=1.943X-0.809$ , 95% confidence limit LCL<sub>50</sub> of 2.15ppm, LCL<sub>90</sub> of 8.83ppm and UCL<sub>50</sub> of 3.14ppm and UCL<sub>90</sub> of 18.17ppm) and acetone extract (LC<sub>50</sub> value 4.1ppm and LC<sub>90</sub> value was 16.471ppm with regression equation  $Y=2.122X-1.3$ , 95% confidence limit LCL<sub>50</sub> of 3.49ppm, LCL<sub>90</sub> of 13.27ppm and UCL<sub>50</sub> of 4.74ppm and UCL<sub>90</sub> of 21.87ppm) showed moderate larvicidal activity against *Ae. aegypti*. The single-way ANOVA result of *Cx. quinquefasciatus* (Table 4.5-4.8) carried out at various concentrations and different replicates revealed significant differences in larval mortality ( $p<0.05$ ).

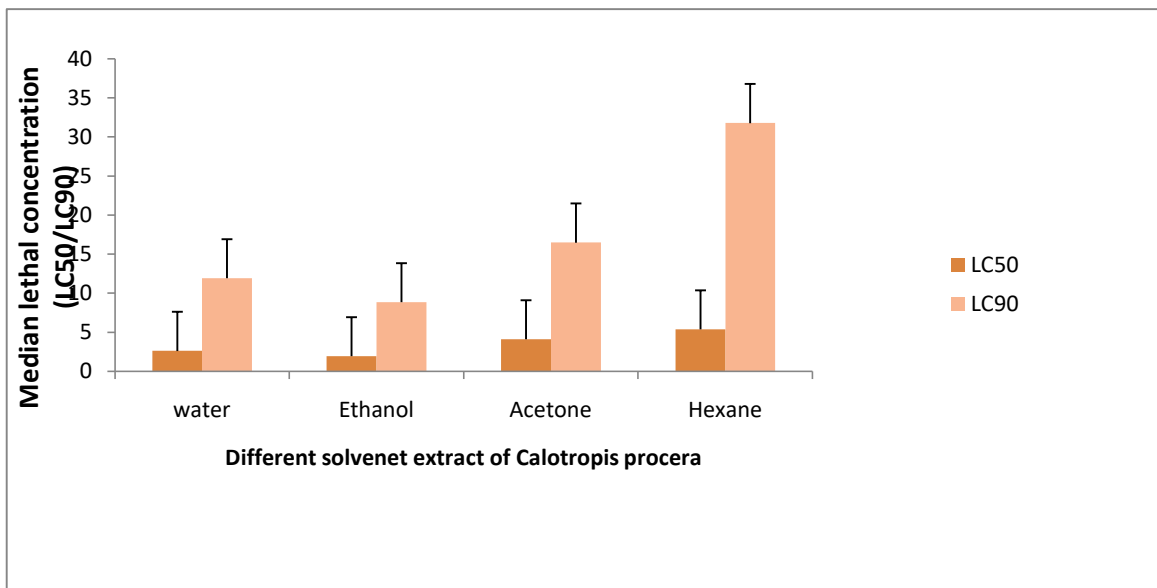
Similar research was conducted by Singh *et al.*, (2005) in which *Ae. aegypti*, the dengue vector, was studied in respect of larvicidal, behavioral, and morphology reaction to the effects of a widespread weed, *C. procera*. The larvicidal efficacy of the *C. procera* leaves towards dengue vector was tested after they had been extracted in hexane. The effectiveness of the *C. procera* leaf extracts in an insecticidal bioassay was determined, with the LC<sub>50</sub> and LC<sub>90</sub> values coming out to be 78.39 as well as 100.60 ppm, correspondingly. After the larvae were exposed to the extracts for extended periods of time, the cytotoxic potential of the extraction increased and the LC<sub>50</sub> values decreased by 2.3%. The *C. procera* extract-assayed larvae showed enhanced wiggling speed and ferocious vertical motions. The most intriguing finding was the persistent self-biting of anal gills that had their own mouth parts, which led to the development of ring-shaped larval structures and suggested a possible effect of the extracts on the neuromuscular system. The lack of cuticular pigment as well as shrinking of the interior cuticle of the anal gills of *Ae. aegypti* larvae were also revealed by morphology studies, suggesting that these were the likely action locations of the *C. procera* leaf extract. It is advised to investigate *C. procera's* potential as a novel larvicide for mosquitoes

management. Evaluation of *C. procera*'s effectiveness at repelling mosquitoes could lead to fruitful research that advances weed management by developing novel anti-mosquito agents. Additionally, Butt *et al.*, (2016) did a study to learn more about the native plant *C. procera*'s larvicidal properties. The plant's leaves as well as stem were utilised to test the insecticidal activity. The substance was extracted with microwave assistance. Various stem and leaf extract percentages (20%, 40%, 60%, 80%, and 100%) were employed. After 24 hours, the larvae started to die. Results indicated that larger levels were more effective than lower amounts. When it came to killing the larvae, it was discovered that leaf extraction was more effective than stem extract. It is determined that this plant's insecticidal qualities of bioactive compounds make all sections of it useful for the control and management of *Ae. aegypti* mosquitoes.

**Table 4.4:** Larval toxicity effect of various solvent extracts of *C. procera* leaves against dengue vector, *Ae. aegypti*

Solvents	LC <sub>50</sub>	LC <sub>90</sub>	Regression equation	95% confidence limit		χ <sup>2</sup>
				LCL LC <sub>50</sub> (LC <sub>90</sub> )	UCL LC <sub>50</sub> (LC <sub>90</sub> )	
<b>Water</b>	2.607	11.903	Y=1.943X-0.809	2.15(8.83)	3.14(18.17)	10.20*
<b>Ethanol</b>	1.923	8.83	Y=1.936X-0.549	1.56(6.58)	2.33(13.39)	8.49*
<b>Acetone</b>	4.1	16.471	Y=2.122X-1.3	3.49(13.27)	4.74(21.87)	8.19*
<b>Hexane</b>	5.364	31.759	Y=1.659X-1.21	4.52(24.35)	6.27(45.30)	21.92*

Control—nil mortality; LCL lower confidence limit, UCL upper confidence limit, χ<sup>2</sup> chi-square value, \*P<0.05 level.



**Figure 4.1** Median lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) of different solvent extracts of *C. procera*

**Table 4.5:** One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of *C. procera* against *Ae. aegypti*

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4241	13	326.1978	634.274	9.1E-56	1.899
Within Groups	28.8	56	0.514286			
Total	4269	69				
SS- Sum of Square	df- degree of freedom		MS- Mean sum of square			
F- Variance ratio						
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Aqueous extract of <i>C. procera</i> showed the statistically significant effects against <i>Ae. aegypti</i> (p<0.05).						

**Table 4.6:** One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of *C. procera* against *Ae. aegypti*

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	3088.183333	11	281	748.6505	1E-49	1.99458
<b>Within Groups</b>	18	48	0.38			
<b>Total</b>	3106.183333	59				
SS- Sum of Square      df- degree of freedom      MS- Mean sum of square						
F- Variance ratio						
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Ethanol extract of <i>C. procera</i> showed the statistically significant effects against <i>Ae. aegypti</i> (p<0.05).						

**Table 4.7:** One way ANOVA analysis between the mortality rate of different replicates and the concentration of acetone extract of *C. procera* against *Ae. aegypti*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	6463	19	340.2	919.397	1.9E-85	1.718
<b>Within Groups</b>	29.6	80	0.37			
<b>Total</b>	6493	99				
SS- Sum of Square      df- degree of freedom      MS- Mean sum of square						
F- Variance ratio						
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Acetone extract of <i>C. procera</i> showed the statistically significant effects against <i>Ae. aegypti</i> (p<0.05).						

**Table 4.8:** One way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of *C. procera* against *Ae. aegypti*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	6345.008	24	264	478.9	2.3E-92	1.62671
<b>Within Groups</b>	55.2	100	0.55			
<b>Total</b>	6400.208	124				
SS- Sum of Square      df- degree of freedom      MS- Mean sum of square						
F- Variance ratio						
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Hexane extract of <i>C. procera</i> showed the statistically significant effects against <i>Ae. aegypti</i> (p<0.05).						

#### 4.5. Larvicidal activity of *C. procera* leaves extract against *Cx. quinquefasciatus*

The larvicidal activities of Aqueous, ethanol, acetone and hexane extracts of leaves parts of *C. procera* are presented below (Table 4.9 and Figure 4.2). It is pertinently noted that, larval mortality was increased with the concentration of extract. Acetone and ethanol extract of the leaves of *C. procera* showed similar larvicidal activity toward *Cx. quinquefasciatus*. The LC<sub>50</sub> value of acetone extract was 4.39ppm and LC<sub>90</sub> value was 17.391ppm with regression equation  $Y=2.144X-1.377$ , 95% confidence limit LCL<sub>50</sub> of 3.397ppm, LCL<sub>90</sub> of 11.357ppm and UCL<sub>50</sub> of 5.83ppm, UCL<sub>90</sub> of 37.549ppm and the ethanol extract showed LC<sub>50</sub> value 5.036ppm, LC<sub>90</sub> value 14.998ppm with regression equation  $Y=2.704X-1.898$ , 95% confidence limit LCL<sub>50</sub> of 4.042ppm, LCL<sub>90</sub> of 10.512ppm and UCL<sub>50</sub> of 6.44ppm and UCL<sub>90</sub> of 28.95ppm. Aqueous extract (LC<sub>50</sub> value 8.178ppm and LC<sub>90</sub> value 30.86ppm with regression equation  $Y=2.222X-2.028$ , 95% confidence limit LCL<sub>50</sub> of 7.19ppm, LCL<sub>90</sub> of 24.26ppm and UCL<sub>50</sub> of 9.32ppm and UCL<sub>90</sub> of 43.05ppm) and hexane extract (LC<sub>50</sub> value 8.52ppm, LC<sub>90</sub> value 31.75ppm with regression equation  $Y=2.244X-2.089$ , 95% confidence limit LCL<sub>50</sub> of 7.51ppm, LCL<sub>90</sub> of 24.91ppm and UCL<sub>50</sub> of 9.72ppm and UCL<sub>90</sub> of 44.52ppm) showed similarly moderate larvicidal activity against *Cx. quinquefasciatus*. The single-way ANOVA result of *Cx. quinquefasciatus* (Table 4.10-4.13) carried out at various concentrations and different replicates revealed significant differences in larval mortality (p<0.05).

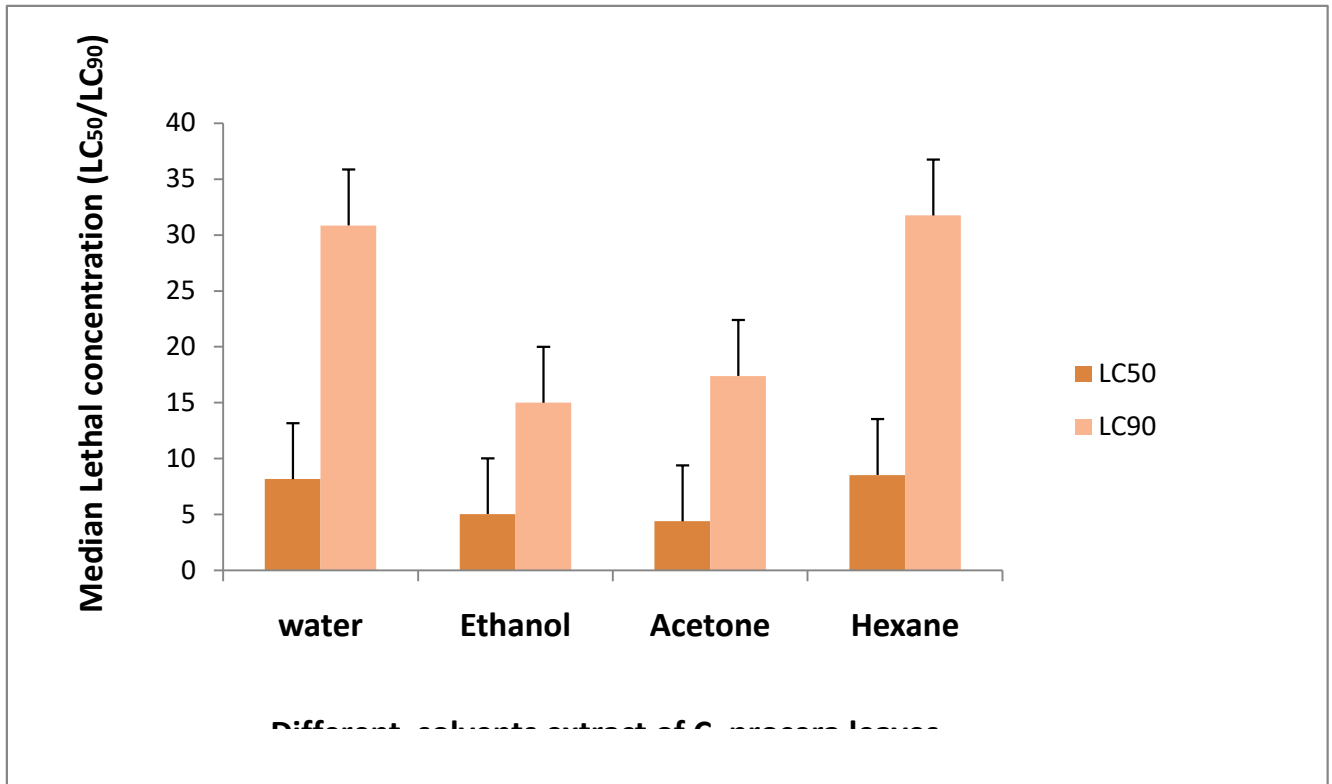
Similarly, following the techniques advised by the WHO, the larvicidal activity of aqueous latex extracts of *C. procera* was evaluated towards the fourth-instar larvae of the filarial vector *Cx. quinquefasciatus*. Dual transform regression modeling was used to quantitatively determine the dose/response mortality connection (Parrotta, 2001).

Furthermore, identical observation was seen by Tahirh *et. al.*, (2013) deals with extract of the *C. procera* and seeks to assess how useful it is and how that affects the mortality of *Cx. quinquefasciatus* larvae. The larvae were evaluated with three different concentrations (*i.e.*, 0.1%, 0.25%, and 0.5%). When comparison to the reference group, the treatment group's mortality was considerably greater. For all levels at 24 hours, the whole extract demonstrated higher larval mortality in shorter time. The percentage death increased as extraction levels were increased, demonstrating a clear correlation between dose and death percentage. The findings indicated that *C. procera* extracts can be employed in mosquitoes control programmes since they have exceptional larvicidal characteristics.

**Table 4.9:** Larval toxicity effect of various solvent extracts of *C. procera* leaves against filaria vector, *Cx. quinquefasciatus*

Solvents	LC <sub>50</sub>	LC <sub>90</sub>	Regression equation	95% confidence limit		$\chi^2$
				LCL LC <sub>50</sub> (LC <sub>90</sub> )	UCL LC <sub>50</sub> (LC <sub>90</sub> )	
<b>Water</b>	8.178	30.86	Y=2.222X-2.028	7.194(24.261)	9.324(43.059)	29.242*
<b>Ethanol</b>	5.036	14.998	Y=2.704X-1.898	4.042(10.512)	6.444(28.959)	23.625*
<b>Acetone</b>	4.39	17.391	Y=2.144X-1.377	3.397(11.357)	5.83(37.549)	22.585*
<b>Hexane</b>	8.528	31.757	Y=2.244X-2.089	7.511(24.91)	9.724(44.524)	24.27*

Control—nil mortality; LCL lower confidence limit, UCL upper confidence limit,  $\chi^2$  chi-square value, \*P<0.05 level.



**Figure 4.2** Median lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) of different solvent extracts of *C. procera* against filaria vector, *Cx. quinquefasciatus*

**Table 4.10** One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of *Calotropis procera* against *Cx. quinquefasciatus*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	6478.922	22	294.4964	1231.531	7.8E-104	1.659134
<b>Within Groups</b>	22	92	0.23913			
<b>Total</b>	6500.922	114				

SS- Sum of Square      df- degree of freedom      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Aqueous extract of *C. procera* showed the statistically significant effects against *Ae. aegypti* (p<0.05).

**Table 4.11** One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of *C. procera* against *Cx. quinquefasciatus*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	4713.147	14	336.6533	953.1481	1.13E-64	1.860242
<b>Within Groups</b>	21.6	60	0.36			
<b>Total</b>	4734.747	74				

SS- Sum of Square      df- degree of freedom      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Ethanol extract of *C. procera* showed the statistically significant effects against *Cx. quinquefasciatus* (p<0.05).

**Table 4.12** One way ANOVA analysis between the mortality rate of different replicates and the concentration of acetone extract of *C. procera* against *Cx. quinquefasciatus*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	4713.147	14	336.6533	953.1481	1.13E-64	1.860242
<b>Within Groups</b>	21.6	60	0.36			
<b>Total</b>	4734.747	74				

SS- Sum of Square      df- degree of freedom      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Acetone extract of *C. procera* showed the statistically significant effects against *Cx. quinquefasciatus* (p<0.05).



**Table 4.13:** One-way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of *C. procera* against *Cx. quinquefasciatus*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	6252.383	22	284.1992	769.0096	1.78E-94	1.659134
<b>Within Groups</b>	34	92	0.369595			
<b>Total</b>	6286.383	114				

SS- Sum of Square      df- degree of freedom      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Hexane extract of *C. procera* showed the statistically significant effects against *Cx. quinquefasciatus* (p<0.05).

#### 4.6. Larvicidal activity of leaves extract of *C. procera* against *An. stephensi*

The 24hrs toxicity effect of aqueous, ethanol, acetone and hexane extracts of *C. procera* leaves were tested against *An. stephensi*. The larvicidal activities of aqueous, ethanol, acetone and hexane extracts of leaves parts of *C. procera* are presented below (Table 4.14 and Figure 4.3). It is noted that, larval mortality was increased with the concentration of extract. Ethanol and hexane extract of the leaves of *C. procera* showed similarity in larvicidal activity against *An. stephensi*. The LC<sub>50</sub> value of ethanol extract was 1.59ppm and LC<sub>90</sub> value was 6.54ppm with regression equation  $Y=2.088X-0.422$ , 95% confidence limit LCL<sub>50</sub> of 1.28ppm, LCL<sub>90</sub> of 4.68ppm and UCL<sub>50</sub> of 1.96ppm, UCL<sub>90</sub> of 10.99ppm while with respect of hexane extract LC<sub>50</sub> value was 1.87ppm, LC<sub>90</sub> value 8.37ppm with regression equation  $Y=1.972X-0.539$ , 95 % confidence limit LCL<sub>50</sub> of 1.51ppm, LCL<sub>90</sub> of 5.71ppm and UCL<sub>50</sub> of 2.36ppm and UCL<sub>90</sub> of 15.45ppm. Aqueous extract (LC<sub>50</sub> value 3.46ppm and LC<sub>90</sub> value 9.69ppm with regression equation  $Y=2.866X-1.547$ , 95 % confidence limit LCL<sub>50</sub> of 3ppm, LCL<sub>90</sub> of 7.87ppm and UCL<sub>50</sub> of 3.97ppm and UCL<sub>90</sub> of 12.99ppm) and acetone extract (LC<sub>50</sub> value 4.06ppm, LC<sub>90</sub> value 14.83ppm with regression equation  $Y=2.279X-1.388$ , 95 % confidence limit LCL<sub>50</sub> of 3.46ppm, LCL<sub>90</sub> of 11.32ppm and UCL<sub>50</sub> of 4.78ppm and UCL<sub>90</sub> of 21.86ppm) showed similarly moderate larvicidal activity against *An. stephensi*. The single-way ANOVA result

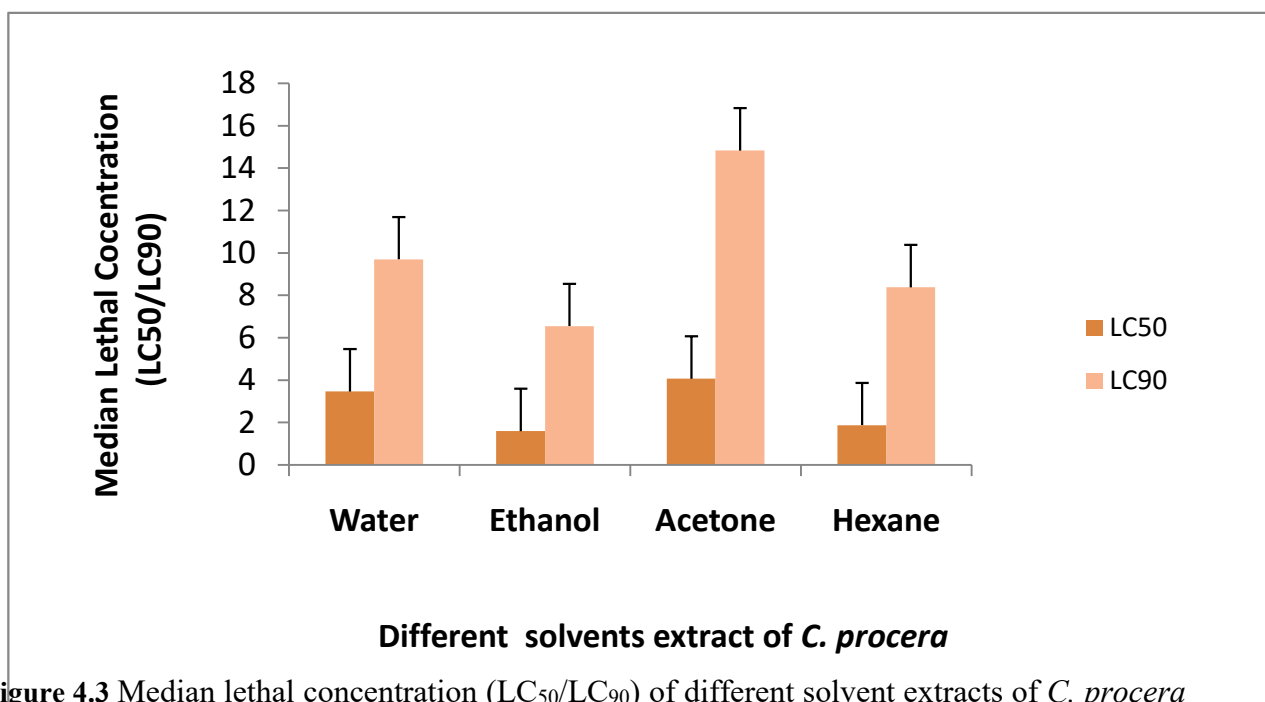
of *Cx. quinquefasciatus* (Table 4.15-4.18) carried out at various concentrations and different replicates revealed significant differences in larval mortality ( $p < 0.05$ ).

These results are supported by Singh *et al.*, (2005) who demonstrated that *C. procera*, a fresh leaf extract, exhibited larvicidal activity against *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* mosquito larvae. However, the same plant's methanolic extracts were more effective larvicides. *C. procera* is a weed that thrives all year long on the Indian subcontinent in arid, dry regions with uncultivated soil. The findings of this study point to milkweed as a promising method for mosquito larvae control.

**Table 4.14:** Larval toxicity effect of various solvent extracts of *C. procera* leaves against malaria vector, *An. stephensi*

Solvents	LC <sub>50</sub>	LC <sub>90</sub>	Regression equation	95% confidence limit		$\chi^2$
				LCL LC <sub>50</sub> (LC <sub>90</sub> )	UCL LC <sub>50</sub> (LC <sub>90</sub> )	
<b>Water</b>	3.464	9.699	Y=2.866X-1.547	3.004(7.874)	3.979(12.992)	10.885*
<b>Ethanol</b>	1.593	6.548	Y=2.088X-0.422	1.289(4.688)	1.965(10.993)	8.332*
<b>Acetone</b>	4.065	14.835	Y=2.27X-1.388	3.468(11.322)	4.786(21.865)	18.117*
<b>Hexane</b>	1.876	8.376	Y=1.972X-0.539	1.511(5.716)	2.365(15.454)	12.904*

Control—nil mortality; LCL lower confidence limit, UCL upper confidence limit,  $\chi^2$  chi-square value, \*P<0.05 level.



**Figure 4.3** Median lethal concentration (LC<sub>50</sub>/LC<sub>90</sub>) of different solvent extracts of *C. procera* leaves against malaria vector, *An. stephensi*

**Table 4.15** One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of *C. procera* against *An. stephensi*

ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	5016.234	13	385.8648	1588.855	7.06E-67	1.899265
Within Groups	13.6	56	0.242857			
Total	5029.843	69				

SS- Sum of Square      df- degree of freedom      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Aqueous extract of *C. procera* showed the statistically significant effects against *An. stephensi* (p<0.05).

**Table 4.16** One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of *C. procera* against *An. stephensi*

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	2948.02	9	327.5578	744.4495	1.28E-41	2.124029
<b>Within Groups</b>	17.6	40	0.44			
<b>Total</b>	2965.62	49				

SS- Sum of Square      df- degree of freedom      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Ethanol extract of *C. procera* showed the statistically significant effects against *An. stephensi* ( $p < 0.05$ ).

**Table 4.17** One way ANOVA analysis between the mortality rate of different replicates and the concentration of acetone extract of *C. procera* against *An. stephensi*

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	4629.947	14	330.7105	885.8316	5.68E-64	1.860242
<b>Within Groups</b>	22.4	60	0.373333			
<b>Total</b>	4652.347	74				

SS- Sum of Square      df- degree of freedom      MS- Mean sum of square  
F- Variance ratio

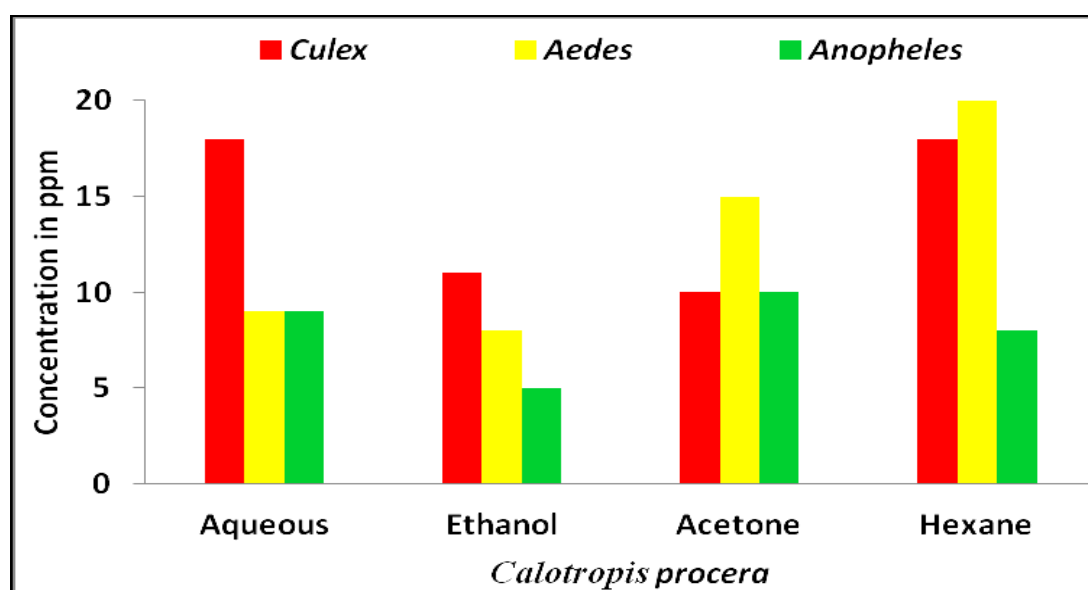
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Acetone extract of *C. procera* showed the statistically significant effects against *An. stephensi* ( $p < 0.05$ ).

**Table 4.18** One-way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of *C. procera* against *An. stephensi*

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	2835.22	9	315.0244	572.7717	2.33E-39	2.124029
<b>Within Groups</b>	22	40	0.55			
<b>Total</b>	2857.22	49				

SS- Sum of Square      df- degree of freedom      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Hexane extract of *C. procera* showed the statistically significant effects against *An. stephensi* ( $p < 0.05$ ).



**Figure 4.4** The 24hrs mortality of mosquito vectors, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* and in various concentrations of different solvent extracts of *C. procera* leaves

Figure (4.4) shows that acetone solvent of *C. procera* showed cent percent mortality at 10ppm against *Culex* while ethanol solvent found more effective against *Aedes* and *Anopheles* at 8ppm and 5ppm respectively.

#### **4.7. Larvicidal activity of *A. marmelos* leaves extract against *Ae. aegypti***

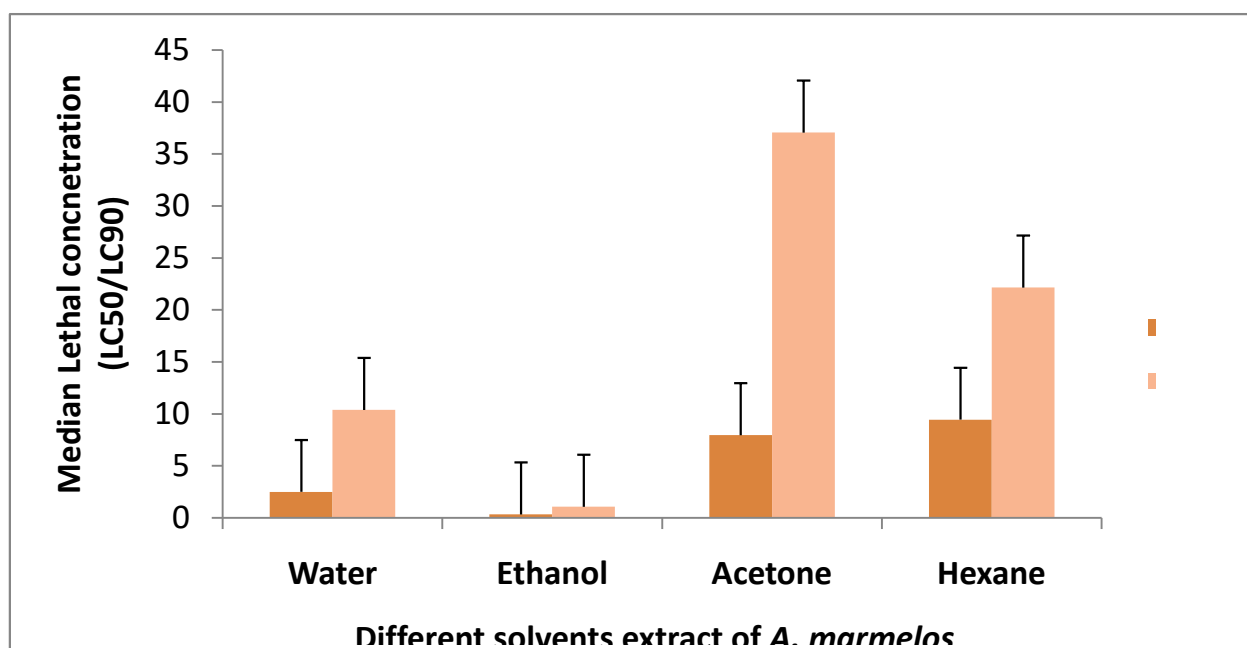
Larvicidal activity of aqueous, ethanol, acetone and hexane extract of *A. marmelos* tested against the fourth instar larvae of *Ae. aegypti* is represented below (Table 4.19 and Figure 4.5). It is pertinently noted that, larval mortality was increased with the concentration of extract. Ethanol extract of the leaves of *A. marmelos* showed the highest larvicidal activity. The LC<sub>50</sub> value of ethanol extract was 0.339ppm and LC<sub>90</sub> value was 1.064ppm with regression equation  $Y=2.582X+1.212$ , 95% confidence limit LCL<sub>50</sub> of 0.218ppm, LCL<sub>90</sub> of 0.807ppm and UCL<sub>50</sub> of 0.463ppm and UCL<sub>90</sub> of 1.801ppm while the hexane extract showed the lowest larvicidal activity with LC<sub>50</sub> value 9.448ppm and LC<sub>90</sub> value was 22.153ppm with regression equation  $Y=3.463X-3.377$ , 95% confidence limit LCL<sub>50</sub> of 7.408ppm, LCL<sub>90</sub> of 16.541ppm and UCL<sub>50</sub> of 11.82ppm and UCL<sub>90</sub> of 39.71ppm. Aqueous extract (LC<sub>50</sub> value 2.497ppm and LC<sub>90</sub> value was 10.39ppm with regression equation  $Y=2.069X-0.822$ , 95% confidence limit LCL<sub>50</sub> of 2.077ppm, LCL<sub>90</sub> of 8.061ppm and UCL<sub>50</sub> of 2.963ppm and UCL<sub>90</sub> of 14.67ppm) and Acetone extract (LC<sub>50</sub> value 7.953ppm and LC<sub>90</sub> value was 37.064ppm with regression equation  $Y=1.917X-1.726$ , 95% confidence limit LCL<sub>50</sub> of 6.917ppm, LCL<sub>90</sub> of 28.619ppm and UCL<sub>50</sub> of 9.142ppm and UCL<sub>90</sub> of 52.67ppm) showed moderate larvicidal activity against *Ae. aegypti*. These results obtained from the One Way ANOVA test (Table 4.20-4.23), concentrations have been found to be statistically significant at  $p<0.05$ .

According to Patil *et al.*, (2011), the highest larval mortality was found in methanol extract of *Aegle marmelos* leaves against *Ae.aegypti* larvae, with LC<sub>50</sub> and LC<sub>90</sub> values of 93.59 and 202.77 ppm, respectively.

**Table 4.19** Larval toxicity effect of various solvent of *A. marmelos* leaves against dengue vector, *Ae. aegypti*.

Solvents	LC <sub>50</sub>	LC <sub>90</sub>	Regression equation	95% confidence limit		χ <sup>2</sup>
				LCL LC <sub>50</sub> (LC <sub>90</sub> )	CL LC <sub>50</sub> (LC <sub>90</sub> )	
<b>Water</b>	2.497	10.39	Y=2.069X-0.822	2.0778(8.061)	2.963(14.67)	12.18*
<b>Ethanol</b>	0.339	1.064	Y=2.582X-1.212	0.218 (0.807)	0.463(1.801)	2.836*
<b>Acetone</b>	7.953	37.064	Y=1.91X-1.726	6.917(28.619)	9.142(52.67)	31.844*
<b>Hexane</b>	9.448	22.153	Y=3.463X-3.377	7.408 (16.541)	11.82(39.71)	122.28*

Control—nil mortality; LCL lower confidence limit, UCL upper confidence limit, χ<sup>2</sup> chi-square value, \*P<0.05 level.



**Figure 4.5:** Median Lethal concentration (LC<sub>50</sub>/LC<sub>90</sub>) of *A. marmelos* leaves against dengue vector, *Ae. aegypti*.

**Table 4.20** One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of *A. marmelos* against *Ae. aegypti*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	5114.986667	14	365.4	741	1.18E-61	1.86024
<b>Within Groups</b>	29.6	60	0.493			
<b>Total</b>	5144.586667	74				
SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square						
F- Variance ratio						
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Aqueous extract of <i>A. marmelos</i> showed the statistically significant effects against <i>Ae. aegypti</i> (p<0.05).						

**Table 4.21** One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of *A. marmelos* against *Ae. aegypti*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	2029.1	5	405.8	497	2.1E-23	2.62065
<b>Within Groups</b>	19.6	24	0.817			
<b>Total</b>	2048.7	29				
SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square						
F- Variance ratio						
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Ethanol extract of <i>A. marmelos</i> showed the statistically significant effects against <i>Ae. aegypti</i> (p<0.05).						



**Table 4.22** One way ANOVA analysis between the mortality rate of different replicates and the concentration of acetone extract of *A. marmelos* against *Ae. aegypti*

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	6864.912	24	286	1212	2.2E-112	1.62671
<b>Within Groups</b>	23.6	100	0.236			
<b>Total</b>	6888.512	124				
SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square F- Variance ratio						
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Acetone extract of <i>A. marmelos</i> showed the statistically significant effects against <i>Ae. aegypti</i> (p<0.05).						

**Table 4.23** One way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of *A. marmelos* against *Ae. aegypti*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	9420.112	24	392.5	1924	2.2E-122	1.62671
<b>Within Groups</b>	20.4	100	0.204			
<b>Total</b>	9440.512	124				
SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square F- Variance ratio						
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Hexane extract of <i>A. marmelos</i> showed the statistically significant effects against <i>Ae. aegypti</i> (p<0.05).						

#### 4.8. Larvicidal activity of *A. marmelos* leaves extract against *Cx. quinquefasciatus*.

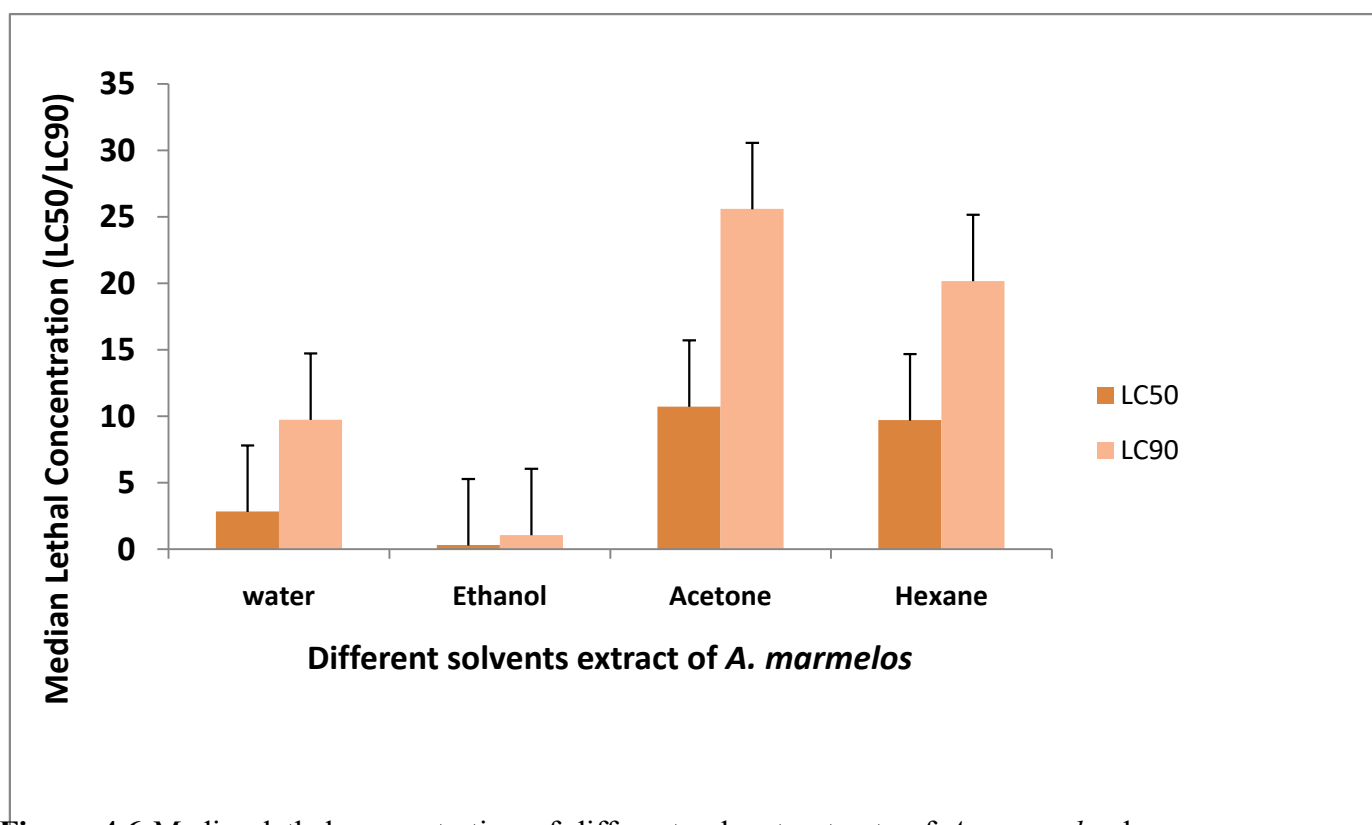
Larvicidal activity of aqueous, ethanol, acetone and hexane extracts of *A. marmelos* against early fourth instar larvae of *Cx. quinquefasciatus* is represented below (Table 4.24 and Figure 4.6). It is observed that larval mortality is increased with the increase rate of concentration. Ethanol extract of *A. marmelos* showed the maximum larvicidal activity against *Cx. quinquefasciatus*. The LC<sub>50</sub> value of ethanol extract was derived to be 0.306ppm and LC<sub>90</sub> value was 1.068ppm with regression coefficient  $Y=2.358X+1.214$ , 95% confidence limit LCL<sub>50</sub> 0.174ppm, LCL<sub>90</sub> 0.793 and UCL<sub>50</sub> 0.407ppm, UCL<sub>90</sub> 1.958ppm while acetone extract showed the lowest larvicidal activity against *Cx. quinquefasciatus*. The LC<sub>50</sub> value of acetone extract was found to be 10.719ppm and LC<sub>90</sub> value was 25.593ppm with regression coefficient  $Y=3.391X-3.493$ , 95% confidence limit LCL<sub>50</sub> 9.857ppm, LCL<sub>90</sub> 22.69ppm and UCL<sub>50</sub> 11.598ppm, UCL<sub>90</sub> 29.804ppm. Furthermore, aqueous and hexane extracts were showed moderate larvicidal activity against *Cx. quinquefasciatus*. The LC<sub>50</sub> value of aqueous extract was 2.827ppm, LC<sub>90</sub> value 9.747ppm with regression coefficient  $Y=2.384X-1.076$ , 95% confidence limit LCL<sub>50</sub> 2.401ppm, LCL<sub>90</sub> 7.908ppm, UCL<sub>50</sub> 3.285ppm, UCL<sub>90</sub> 12.843ppm and LC<sub>50</sub> value of hexane extract was 9.701ppm, LC<sub>90</sub> value 20.172ppm with regression coefficient  $Y=4.031X-3.978$ , 95% confidence limit LCL<sub>50</sub> 8.958ppm, LCL<sub>90</sub> 17.976ppm, UCL<sub>50</sub> 10.469ppm, UCL 23.417ppm. These results (Table 4.25-4.28) have been proved statistically significant  $p<0.05$  by the One Way ANOVA test.

Similar findings were made in a study conducted by Dass *et al.*, (2010) that looked at plant components as possible pesticide substitutes. Leaf extract from *A. marmelos* is tested against *Cx. quinquefasciatus* pupa, 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> instars to see whether it has larvicidal properties. The LC<sub>50</sub> value for *A. marmelos* was determined to be 91.52 mg/ml, 105.16 mg/ml, 151.43 mg/ml, and pupa, respectively, for the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> instars. The adult emergence data from this study shows that, regardless of the plant extracts investigated, adult emergence decreases as a result of concentration. Similarly, *A. marmelos* extracts in water, ethanol, acetone, and hexane were tested for their ability to kill early fourth instar *Cx. quinquefasciatus* larvae. It has been noted that larval mortality rises as concentration increases. *A. marmelos* ethanol extract had the strongest larvicidal effect on *Cx. quinquefasciatus*.

**Table 4.24** Larval toxicity effect of various solvent extracts of *A. marmelos* leaves against filaria vector, *Cx. quinquefasciatus*.

Solvents	LC <sub>50</sub>	LC <sub>90</sub>	Regression equation	95% confidence limit		χ <sup>2</sup>
				LCL LC <sub>50</sub> (LC <sub>90</sub> )	UCL LC <sub>50</sub> (LC <sub>90</sub> )	
<b>Water</b>	2.827	9.747	Y=2.384X-1.076	2.401(7.908)	3.285(12.843)	8.653*
<b>Ethanol</b>	0.306	1.068	Y=2.358X+1.214	0.174(0.793)	0.407(1.958)	2.727*
<b>Acetone</b>	10.719	25.593	Y=3.391X-3.493	9.857(22.69)	11.598(29.804)	17.884*
<b>Hexane</b>	9.701	20.172	Y=4.031X-3.978	8.958(17.976)	10.469(23.417)	22.961*

Control—nil mortality; LCL lower confidence limit, UCL upper confidence limit, χ<sup>2</sup> chi-square value, \*P<0.05 level.



**Figure 4.6** Median lethal concentration of different solvent extracts of *A. marmelos* leaves against filaria vector, *Cx. quinquefasciatus*.

**Table 4.25:** One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of *A. marmelos* against *Cx. quinquefasciatus*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	5943.488	15	396.2325	1132.093	1.57E-71	1.825586
<b>Within Groups</b>	22.4	64	0.35			
<b>Total</b>	5965.888	79				
SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square						
F- Variance ratio						
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Aqueous extract of <i>A. marmelos</i> showed the statistically significant effects against <i>Cx. quinquefasciatus</i> (p<0.05).						

**Table 4.26:** One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of *A. marmelos* against *Cx. quinquefasciatus*

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	1967.067	5	393.4133	907.8769	1.61E-26	2.620654
<b>Within Groups</b>	10.4	24	0.433333			
<b>Total</b>	1977.467	29				
SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square						
F- Variance ratio						
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Ethanol extract of <i>A. marmelos</i> showed the statistically significant effects against <i>Cx. quinquefasciatus</i> (p<0.05).						

**Table 4.27** One way ANOVA analysis between the mortality rate of different replicates and the concentration of acetone extract of *A. marmelos* against *Cx. quinquefasciatus*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	11492.43	29	396.2908	982.5392	4.3E-129	1.562071
<b>Within Groups</b>	48.4	120	0.403333			
<b>Total</b>	11540.83	149				

SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Acetone extract of *A. marmelos* showed the statistically significant effects against *Cx. quinquefasciatus* (p<0.05).

**Table 4.28** One way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of *A. marmelos* against *Cx. quinquefasciatus*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	9624	24	401	1542.308	1.4E-117	1.626708
<b>Within Groups</b>	26	100	0.26			
<b>Total</b>	9650	124				

SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Hexane extract of *A. marmelos* showed the statistically significant effects against *Cx. quinquefasciatus* (p<0.05).

#### 4.9. Larvicidal activity of *A. marmelos* leaves extract against *An. stephensi*

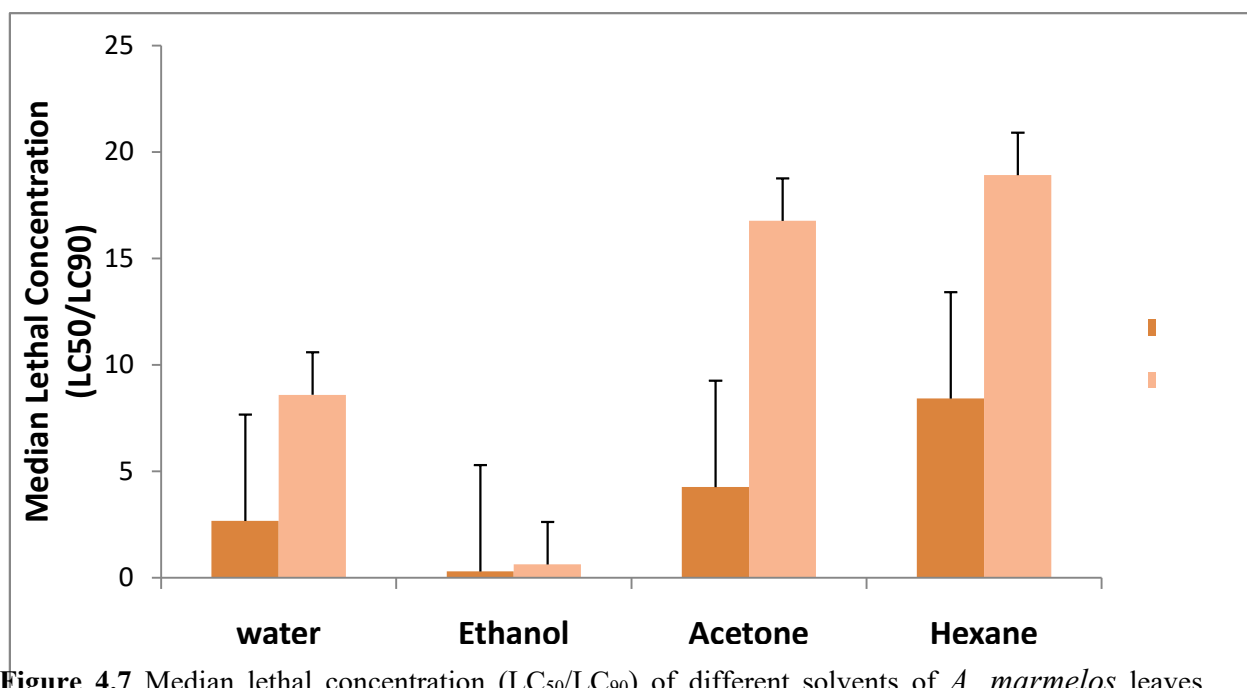
Larvicidal activity of ethanol, aqueous, hexane and acetone extract of *A. marmelos* tested against the 4<sup>th</sup> instar larvae of *An. stephensi* is represented below (Table 4.29 and Figure 4.7). It is pertinently noted that, larval mortality was increased with the concentration of extract. Ethanol extract of the leaves of *A. marmelos* showed the highest larvicidal activity against *An. stephensi*. The LC<sub>50</sub> value of ethanol extract was 0.296ppm and LC<sub>90</sub> value was 0.631ppm with regression equation  $Y=3.901X+2.062$ , 95% confidence limit LCL<sub>50</sub> of 0.213ppm, LCL<sub>90</sub> of 0.513ppm and UCL<sub>50</sub> of 0.362ppm and UCL<sub>90</sub> of 0.9ppm while the hexane extract showed the lowest larvicidal activity with LC<sub>50</sub> value 8.419ppm and LC<sub>90</sub> value was 18.915ppm with regression equation  $Y=3.645X-3.373$ , 95% confidence limit LCL<sub>50</sub> of 6.214ppm, LCL<sub>90</sub> of 13.017ppm and UCL<sub>50</sub> of 11.831ppm and UCL<sub>90</sub> of 54.95ppm. Aqueous extract (LC<sub>50</sub> value 2.671ppm and LC<sub>90</sub> value was 8.596ppm with LCL<sub>50</sub> of 2.276ppm with regression equation  $Y=2.525X-1.078$ , 95% confidence limit LCL<sub>90</sub> of 6.999ppm and UCL<sub>50</sub> of 3.099ppm and UCL<sub>90</sub> of 11.29ppm) and Acetone extract (LC<sub>50</sub> value 4.267ppm and LC<sub>90</sub> value was 16.773ppm with regression equation  $Y=2.156X-1.359$ , 95% confidence limit LCL<sub>50</sub> of 3.659ppm, LCL<sub>90</sub> of 13.539ppm and UCL<sub>50</sub> of 4.924ppm and UCL<sub>90</sub> of 22.204ppm) showed moderate larvicidal activity against *An. stephensi*. These results obtained from the One Way ANOVA test (Table 4.30-4.33), concentrations have been found to be statistically significant at  $p<0.05$ .

Similar results were obtained from an experiment conducted by Rathy *et.al.*, (2015) in which *A. marmelos* showed strong larvicidal activity against 4<sup>th</sup> instar larvae of *An. stephensi*. Sukumar *et. al.*, (1991) have pointed out that the most promising mosquito control agents of botanical origin are from the families of Rutaceae (*A. marmelos*). The present finding is a new addition to the list of plants being reported to have larvicidal properties.

**Table 4.29:** Larval toxicity effect of various solvent extracts of *A. marmelos* leaves against malaria vector, *An. stephensi*

Solvents	LC <sub>50</sub>	LC <sub>90</sub>	Regression equation	95% confidence limit		χ <sup>2</sup>
				LCL LC <sub>50</sub> (LC <sub>90</sub> )	UCL LC <sub>50</sub> (LC <sub>90</sub> )	
<b>Water</b>	2.671	8.596	Y=2.525X-1.078	2.276 (6.999)	3.099(11.29)	9.496*
<b>Ethanol</b>	0.296	0.631	Y=3.901X+2.062	0.213(0.513)	0.362(0.9)	0.772*
<b>Acetone</b>	4.267	16.773	Y=2.156X-1.359	3.659(13.539)	4.924(22.204)	18.376*
<b>Hexane</b>	8.419	18.915	Y=3.645X-3.373	6.214(13.017)	11.831(54.95)	121.762*

Control—nil mortality; LCL lower confidence limit, UCL upper confidence limit, χ<sup>2</sup> chi-square value, \*P<0.05 level.



**Figure 4.7** Median lethal concentration (LC<sub>50</sub>/LC<sub>90</sub>) of different solvents of *A. marmelos* leaves against malaria vector, *An. stephensi*.

**Table 4.30** One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of *A. marmelos* against *An. stephensi*

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	5729.467	14	409.2476	1278.899	9.84E-69	1.860242
<b>Within Groups</b>	19.2	60	0.32			
<b>Total</b>	5748.667	74				

SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Aqueous extract of *A. marmelos* showed the statistically significant effects against *An. stephensi* (p<0.05).

**Table 4.31** One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of *A. marmelos* against *An. stephensi*

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	2114.24	4	528.56	2642.8	6.33E-27	2.866081
<b>Within Groups</b>	4	20	0.2			
<b>Total</b>	2118.24	24				

SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Ethanol extract of *A. marmelos* showed the statistically significant effects against *An. stephensi* (p<0.05).



**Table 4.32** One way ANOVA analysis between the mortality rate of different replicates and the concentration of acetone extract of *A. marmelos* against *An. stephensi*

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	7062.64	19	371.7179	906.629	3.38E-85	1.718026
<b>Within Groups</b>	32.8	80	0.41			
<b>Total</b>	7095.44	99				

SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square  
F- Variance ratio

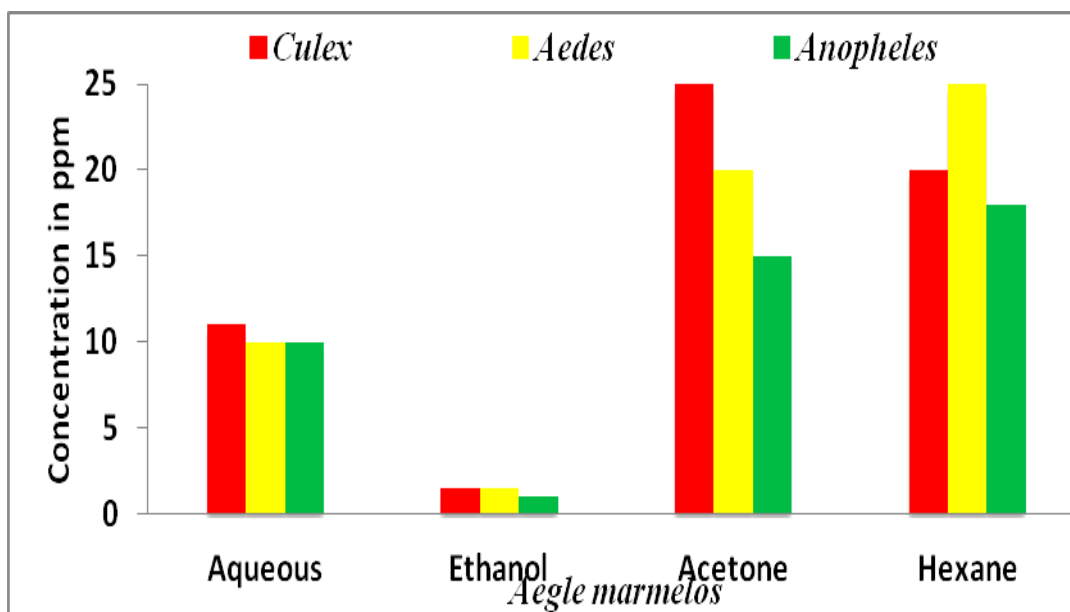
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Acetone extract of *A. marmelos* showed the statistically significant effects against *An. stephensi* (p<0.05).

**Table 4.33** One way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of *A. marmelos* against *An. stephensi*

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	6814.59	19	358.6626	1086.856	2.48E-88	1.718026
<b>Within Groups</b>	26.4	80	0.33			
<b>Total</b>	6840.99	99				

SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Hexane extract of *A. marmelos* showed the statistically significant effects against *An. stephensi* (p<0.05).



**Figure 4.8** The 24hrs mortality of mosquito vectors, *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* in various concentration of different solvent extracts of *A. marmelos* leaves

In case of *A. marmelos* (Figure 4.8), as compare to aqueous, ethanol, acetone and hexane solvent, ethanol solvent was revealed 100% mortality at low concentration against *Culex* (1.5ppm), *Aedes* (1.5ppm) and *Anopheles* (1.0ppm) larvae.

#### 4.10. Larvicidal activity of *A. americana* leaves extract against *Ae. aegypti*

Larvicidal activity of aqueous, ethanol, acetone and hexane extract of *A. americana* tested against the fourth instar larvae of *An. stephensi* is represented below (Table 4.34 and Figure 4.9). It is pertinently noted that, larval mortality was increased with the concentration of extract. Aqueous extract of the leaves of *A. americana* showed the highest larvicidal activity against *Ae. aegypti*. The LC<sub>50</sub> value of aqueous extract was 0.418ppm and LC<sub>90</sub> value was 1.083ppm with regression equation  $Y=1.929X-0.807$ , 95% confidence limit LCL<sub>50</sub> of 0.195ppm, LCL<sub>90</sub> of 0.905ppm and UCL<sub>50</sub> of 0.555ppm and UCL<sub>90</sub> of 1.452ppm while the acetone extract showed the lowest larvicidal activity with LC<sub>50</sub> value 14.245ppm and LC<sub>90</sub> value was 43.811ppm with regression equation  $Y=2.627X-3.03$ , 95% confidence limit LCL<sub>50</sub> of 13.056ppm, LCL<sub>90</sub> of 38.696ppm and UCL<sub>50</sub> of 15.426ppm and UCL<sub>90</sub> of 51.057ppm. Hexane extract (LC<sub>50</sub> value 9.487ppm and LC<sub>90</sub> value was 22.51ppm with regression equation  $Y=3.415X-3.337$ , 95% confidence limit LCL<sub>50</sub> of 7.556ppm, LCL<sub>90</sub> of 16.953ppm and UCL<sub>50</sub> of 11.725ppm and UCL<sub>90</sub> of 38.84ppm) and ethanol extract (LC<sub>50</sub> value 12.595ppm

and LC<sub>90</sub> value was 26.532ppm with regression Y=3.961X-4.358, 95% confidence limit LCL<sub>50</sub> of 11.729ppm, LCL<sub>90</sub> of 23.753ppm and UCL<sub>50</sub> of 13.498ppm and UCL<sub>90</sub> of 30.563ppm) showed moderate larvicidal activity against *An. stephensi*. These results obtained from the One Way ANOVA test (Table 4.35-4.38), concentrations have been found to be statistically significant at p<0.05.

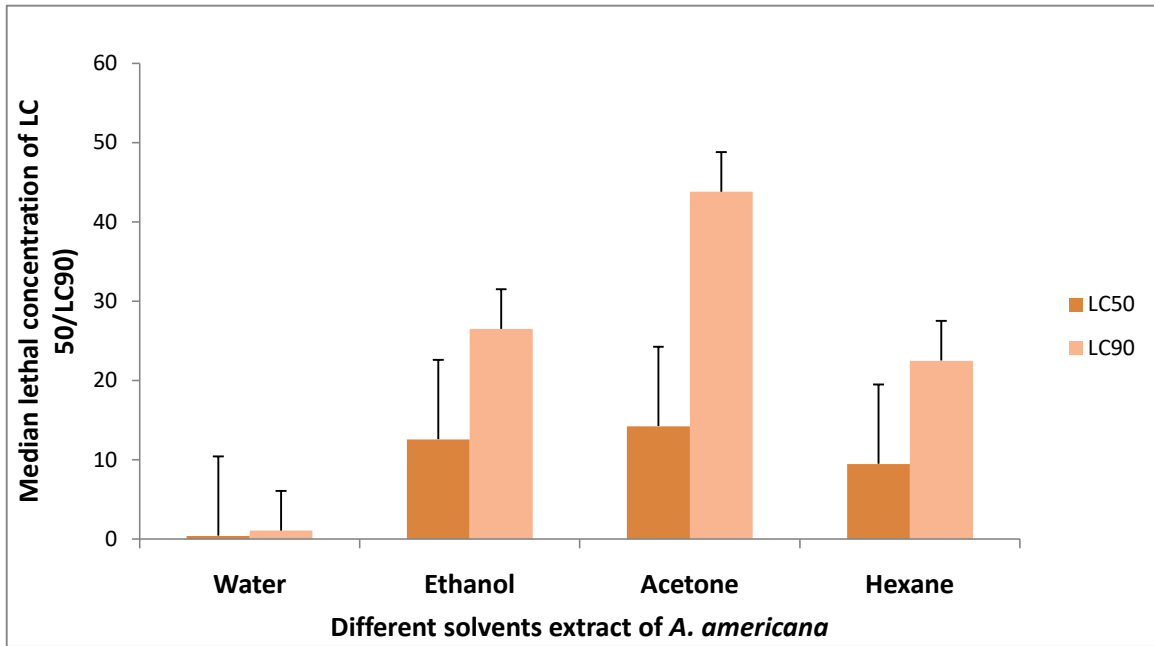
The result is supported by a test that was done by Torres *et al.*, (2015) using leaf extract of *A. americana* against this mosquito species, larvae died completely within 24 to 48 hours at a concentration of 0.08%, whereas stage first larvae died completely at lower concentrations -0.0032% for *Ae. aegypti*. When exposed to 4<sup>th</sup> instar larvae at room temperature for 24 hours, the greatest dilution of the seed extract (1:200) results in 100% larval mortality for *Aedes* mosquito. The control group's comparative mortality was incredibly low.

Dharmshaktu *et al.*, (1987) also concluded 100% mortality of *A. americana* at lower concentration of 0.0032% against *Ae. aegypti* first instar larvae within 24-48 hrs.

**Table 4.34** Larval toxicity effect of different solvents of *A. americana* leaves against dengue vector, *Ae. aegypti*.

Solvents	LC <sub>50</sub>	LC <sub>90</sub>	Regression equation	95% confidence limit		χ <sup>2</sup>
				LCL LC <sub>50</sub> (LC <sub>90</sub> )	UCL LC <sub>50</sub> (LC <sub>90</sub> )	
<b>Water</b>	0.418	1.083	Y=1.929X-0.807	0.195 (0.905)	0.555(1.452)	0.782*
<b>Ethanol</b>	12.595	26.532	Y=3.961X-4.358	11.729(23.753)	13.498(30.563)	32.788*
<b>Acetone</b>	14.245	43.811	Y=2.627X-3.03	13.056(38.696)	15.426(51.057)	30.701*
<b>Hexane</b>	9.487	22.51	Y=3.415X-3.337	7.556(16.953)	11.725(38.84)	108.184*

Control—nil mortality; LCL lower confidence limit, UCL upper confidence limit, χ<sup>2</sup> chi-square value, \*P<0.05 level.



**Figure 4.9** Median Lethal concentration of different solvents of *A. americana* leaves against dengue vector, *Ae. aegypti*.

**Table 4.35** One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of *A. americana* against *Ae. Aegypti*

ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	1999.766667	5	400	706	3.24E-25	2.62065
Within Groups	13.6	24	0.567			
Total	2013.366667	29				

SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Aqueous extract of *A. americana* showed the statistically significant effects against *Ae. aegypti* ( $p < 0.05$ ).

**Table 4.36** One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of *A. americana* against *Ae. aegypti*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	11305.09333	29	389.8	1917	1.9E-146	1.56207
<b>Within Groups</b>	24.4	120	0.203			
<b>Total</b>	11329.49333	149				
SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square						
F- Variance ratio						
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Ethanol extract of <i>A. americana</i> showed the statistically significant effects against <i>Ae. aegypti</i> (p<0.05).						

**Table 4.37** One way ANOVA analysis between the mortality rate of different replicates and the concentration of acetone extract of *A. americana* against *Ae. aegypti*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	14285.66222	44	324.7	1074	2.8E-196	1.44395
<b>Within Groups</b>	54.4	180	0.302			
<b>Total</b>	14340.06222	224				
SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square						
F- Variance ratio						
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Acetone extract of <i>A. americana</i> showed the statistically significant effects against <i>Ae. aegypti</i> (p<0.05).						

**Table 4.38** One way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of *A. americana* against *Ae. aegypti*

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	9330.752	24	388.8	1767	1.5E-120	1.62671
<b>Within Groups</b>	22	100	0.22			
<b>Total</b>	9352.752	124				

SS- Sum of Square      df- degree of freedom      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Hexane extract of *A. americana* showed the statistically significant effects against *Ae. aegypti* ( $p < 0.05$ ).

#### 4.11. Larvicidal activity of *A. americana* leaves extract against *Cx. quinquefasciatus*

Larvicidal activity of aqueous, ethanol, acetone and hexane extract of *A. americana* tested against the fourth instar larvae of *Cx. quinquefasciatus* is represented in Table 4.39 and Figure 4.10. It is pertinently noted that, larval mortality was increased with the concentration of extract. Aqueous extract of the leaves of *A. americana* showed the highest larvicidal activity against filaria vector, *Cx. quinquefasciatus*. The LC<sub>50</sub> value of aqueous extract was 0.212ppm and LC<sub>90</sub> value was 0.586ppm with regression equation  $Y=2.909X+1.957$ , 95 % confidence limit LCL<sub>50</sub> of 0.093ppm, LCL<sub>90</sub> of 0.451ppm and UCL<sub>50</sub> of 0.293ppm and UCL<sub>90</sub> of 0.971ppm while the acetone extract showed the lowest larvicidal activity with LC<sub>50</sub> value 13.913ppm and LC<sub>90</sub> value was 40.549ppm with regression equation  $Y=2.759X-3.154$ , 95 % confidence limit LCL<sub>50</sub> of 12.769ppm, LCL<sub>90</sub> of 35.581ppm and UCL<sub>50</sub> of 15.065ppm and UCL<sub>90</sub> of 47.794ppm. Ethanol extract (LC<sub>50</sub> value 11.709ppm and LC<sub>90</sub> value was 24.5ppm with regression equation  $Y=3.997X-4.271$ , 95 % confidence limit LCL<sub>50</sub> of 10.005ppm, LCL<sub>90</sub> of 19.305ppm and UCL<sub>50</sub> of 13.86ppm and UCL<sub>90</sub> of 37.942ppm) and hexane extract (LC<sub>50</sub> value 12.797ppm and LC<sub>90</sub> value was 43.242ppm with regression equation  $Y=2.423X-2.683$ , 95 % confidence limit LCL<sub>50</sub> of 12.769ppm, LCL<sub>90</sub> of 35.581ppm and UCL<sub>50</sub> of 15.065ppm and UCL<sub>90</sub> of 47.794ppm) showed moderate larvicidal activity

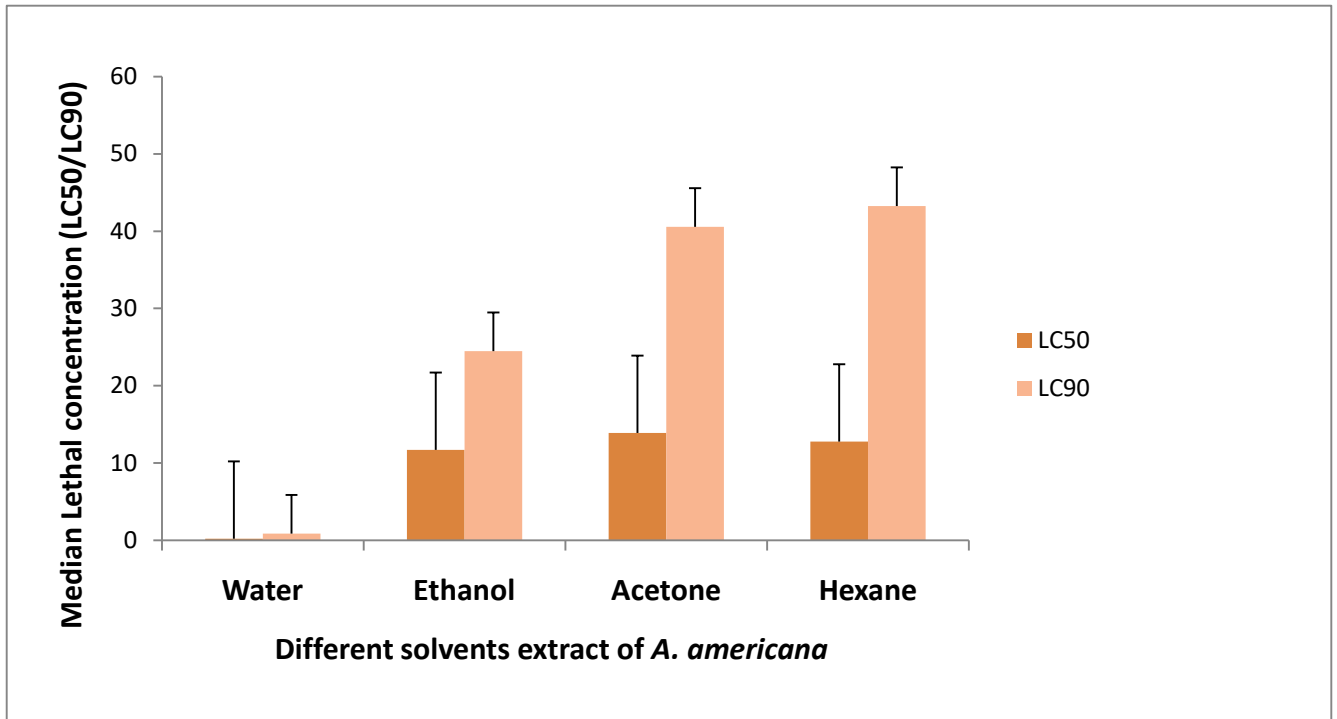
against *Cx. quinquefasciatus*. These results obtained from the One Way ANOVA test (Table 4.40-4.43), concentrations have been found to be statistically significant at  $p < 0.05$ .

This output is supported by Maazoun *et.al.*, (2019) who explored that the leaf extract of *A. americana* tested against mosquito species, the fourth-stage Culex larvae died completely at a dosage of 0.08% within 24–48 hours, whereas the stage first larvae died completely at lower concentrations—at 0.016% for *Cx. quinquefasciatus*—and more quickly. When fourth instar larvae of Culex mosquito were treated at the room temperature for 24 hours, the greatest dilution of the seed extract (1:200) produced a mortality of 100% against 56% of the species.

**Table 4.39** Larval toxicity effect of various solvent extracts of *A. americana* leaves against filaria vector, *Cx. quinquefasciatus*.

Solvents	LC <sub>50</sub>	LC <sub>90</sub>	Regression equation	95% confidence limit		$\chi^2$
				LCL LC <sub>50</sub> (LC <sub>90</sub> )	UCL LC <sub>50</sub> (LC <sub>90</sub> )	
<b>Water</b>	0.212	0.586	Y=2.909X+1.957	0.093 (0.451)	0.293(0.971)	1.052*
<b>Ethanol</b>	11.709	24.5	Y=3.997X-4.271	10.005(19.305)	13.86(37.942)	78.256*
<b>Acetone</b>	13.913	40.549	Y=2.759X-3.154	12.769(35.581)	15.065(47.794)	31.261*
<b>Hexane</b>	12.797	43.242	Y=2.423X-2.683	11.611(36.462)	14.057(53.917)	26.463*

Control—nil mortality; LCL-lower confidence limit, UCL- upper confidence limit,  $\chi^2$  chi-square value, \*P<0.05 level.



**Figure 4.10** Median Lethal concentration (LC<sub>50</sub>/LC<sub>90</sub>) of different solvents of *A. americana* leaves against filaria vector, *Cx. quinquefasciatus*

**Table 4.40** One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of *A. americana* against *Cx. quinquefasciatus*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	1995.84	4	499	2772	3.93E-27	2.86608
<b>Within Groups</b>	3.6	20	0.18			
<b>Total</b>	1999.44	24				

SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square

F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Aqueous extract of *A.americana* showed the statistically significant effects against *Cx. quinquefasciatus* (p<0.05).



**Table 4.41** One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of *A. americana* against *Cx. quinquefasciatus*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	8864.672	24	369	1489.36	8E-117	1.62671
<b>Within Groups</b>	24.8	100	0.25			
<b>Total</b>	8889.472	124				
SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square F- Variance ratio						
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Ethanol extract of <i>A. americana</i> showed the statistically significant effects against <i>Cx. quinquefasciatus</i> (p<0.05).						

**Table 4.42** One way ANOVA analysis between the mortality rate of different replicates and the concentration of acetone extract of *A. americana* against *Cx. quinquefasciatus*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	12299.9	39	315	1157.365	3E-177	1.47507
<b>Within Groups</b>	43.6	160	0.27			
<b>Total</b>	12343.5	199				
SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square F- Variance ratio						
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Acetone extract of <i>A. americana</i> showed the statistically significant effects against <i>Cx. quinquefasciatus</i> (p<0.05).						

**Table 4.43** One way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of *A. americana* against *Cx. quinquefasciatus*

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	9882.377	34	290.6582	933.306	8.8E-149	1.513376
<b>Within Groups</b>	43.6	140	0.311429			
<b>Total</b>	9925.977	174				

SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Hexane extract of *A. americana* showed the statistically significant effects against *Cx. quinquefasciatus* (p<0.05).

#### 4.12. Larvicidal activity of *A. americana* leaves extract against *An. stephensi*

Larvicidal activity of aqueous, ethanol, acetone and hexane extract of *A. americana* tested against the fourth instar larvae of *An. stephensi* is represented in Table 4.44 and Figure 4.11. It is pertinently noted that, larval mortality was increased with the concentration of extract. Aqueous extract of the leaves of *A. americana* showed the highest larvicidal activity against filaria vector, *Cx. quinquefasciatus*. The LC<sub>50</sub> value of aqueous extract was 0.212ppm and LC<sub>90</sub> value was 0.668ppm with regression equation  $Y=2.575X+1.732$ , 95% confidence limit LCL<sub>50</sub> of 0.08ppm, LCL<sub>90</sub> of 0.502ppm and UCL<sub>50</sub> of 0.301ppm and UCL<sub>90</sub> of 1.256ppm while the acetone extract showed the lowest larvicidal activity with LC<sub>50</sub> value 12.677ppm and LC<sub>90</sub> value was 45.638ppm with regression equation  $Y=2.304X-2.541$ , 95% confidence limit LCL<sub>50</sub> of 11.472ppm, LCL<sub>90</sub> of 38.995ppm and UCL<sub>50</sub> of 13.906ppm and UCL<sub>90</sub> of 55.698ppm. Hexane extract (LC<sub>50</sub> value 1.974ppm and LC<sub>90</sub> value was 10.799ppm with regression equation  $Y=1.737X-0.513$ , 95% confidence limit LCL<sub>50</sub> of 1.579ppm, LCL<sub>90</sub> of 8.034ppm and UCL<sub>50</sub> of 2.409ppm and UCL<sub>90</sub> of 16.291ppm) and ethanol extract (LC<sub>50</sub> value 11.806ppm and LC<sub>90</sub> value was 24.527ppm with regression equation  $Y=4.036X-4.327$ , 95% confidence limit LCL<sub>50</sub> of 10.009ppm, LCL<sub>90</sub> of 19.157ppm and UCL<sub>50</sub> of 14.121ppm and UCL<sub>90</sub> of 39.252ppm) showed moderate larvicidal activity against *An. stephensi*. These

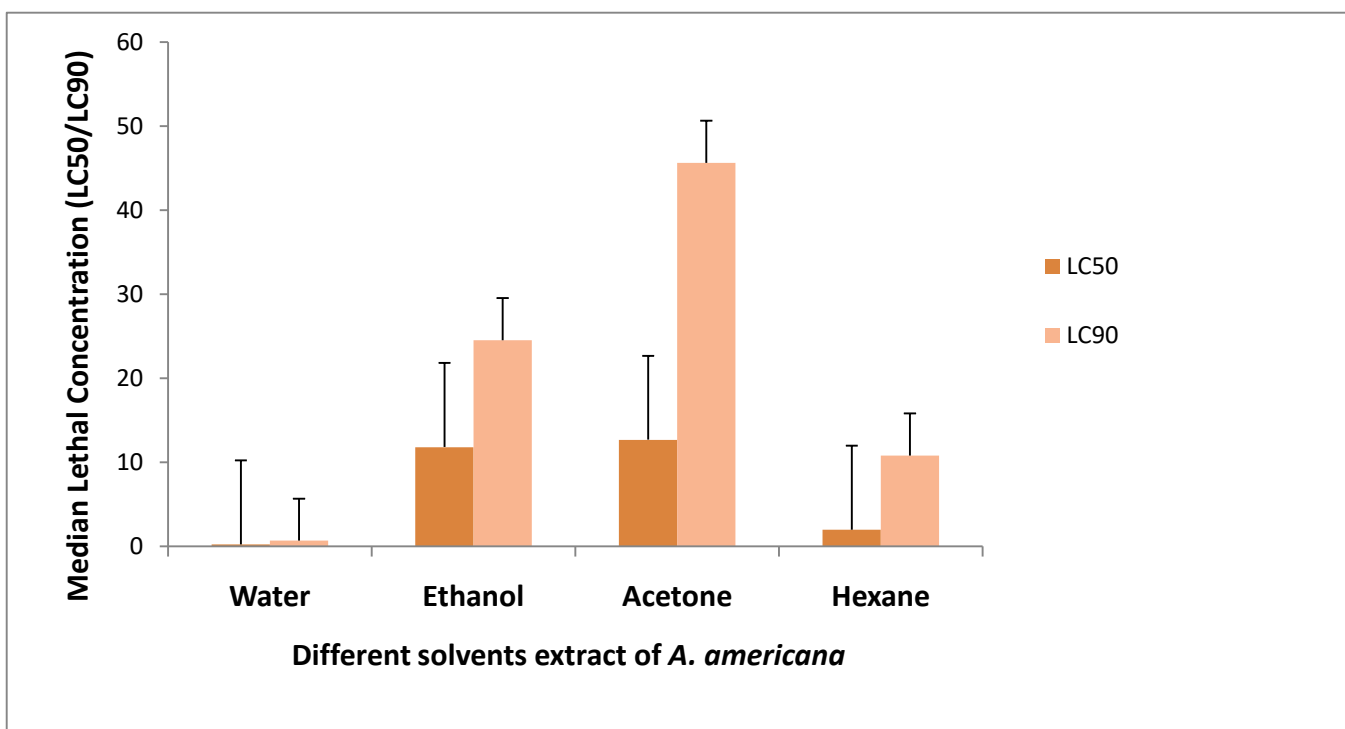
results (Table 4.45-4.48) obtained from the One Way ANOVA test, concentrations have been found to be statistically significant at  $p < 0.05$ .

This result was supported by study carried out by Dey *et al.*, (2013) study was conducted to evaluate the larvicidal efficacy of solvents extracts from *A. americana* leaf as well as flower towards mosquito larvae in their fourth instar at doses of 100mg/ml, 150 mg/ml, 200 mg/ml, 250mg/ml, and 300 mg/ml. Additionally, phytochemical testing was done following established protocols. According to their research, *A. americana* leaf extracts in chloroform, acetone and chloroform as well as ethanol may be utilised as an environmentally acceptable method to suppress *An. stephensi* larvae in its fourth instar. (Govindarajan *et al.*, 2011).

**Table 4.44:** Larval toxicity effect of various solvent extracts of *A. americana* leaves against malaria vector, *An. stephensi*.

Solvents	LC <sub>50</sub>	LC <sub>90</sub>	Regression equation	95% confidence limit		$\chi^2$
				LCL LC <sub>50</sub> (LC <sub>90</sub> )	UCL LC <sub>50</sub> (LC <sub>90</sub> )	
<b>Water</b>	0.212	0.668	Y=2.575X+1.732	0.08 (0.502)	0.301(1.256)	1.903*
<b>Ethanol</b>	11.806	24.527	Y=4.036X-4.327	10.009(19.157)	14.121(39.252)	87.04*
<b>Acetone</b>	12.677	45.638	Y=2.304X-2.541	11.472(38.995)	13.906(55.698)	19.644*
<b>Hexane</b>	1.974	10.799	Y=1.737X-0.513	11.579(8.034)	2.409(16.291)	6.302*

Control—nil mortality; within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT LCL lower confidence limit, UCL upper confidence limit,  $\chi^2$  chi-square value, \*P<0.05 level.



**Figure 4.11** Median Lethal concentration (LC<sub>50</sub>/LC<sub>90</sub>) of different solvents extract of *A. americana* leaves against malaria vector, *An. stephensi*.

**Table 4.45** One way ANOVA analysis between the mortality rate of different replicates and the concentration of aqueous extract of *A. americana* against *An. stephensi*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	1914.96	4	478.74	1841.308	2.33E-25	2.866081
<b>Within Groups</b>	5.2	20	0.26			
<b>Total</b>	1920.16	24				
SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square						
F- Variance ratio						
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Aqueous extract of <i>A. americana</i> showed the statistically significant effects against <i>An. stephensi</i> (p<0.05).						

**Table 4.46** One way ANOVA analysis between the mortality rate of different replicates and the concentration of ethanol extract of *A. americana* against *An. stephensi*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	8757.392	24	364.8913	1249.628	4.8E-113	1.626708
<b>Within Groups</b>	29.2	100	0.292			
<b>Total</b>	8786.592	124				

SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Ethanol extract of *A. americana* showed the statistically significant effects against *An. stephensi* (p<0.05).

**Table 4.47** One way ANOVA analysis between the mortality rate of different replicates and the concentration of acetone extract of *A. americana* against *An. stephensi*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	11162.78	39	286.2251	854.4034	9.6E-167	1.475066
<b>Within Groups</b>	53.6	160	0.335			
<b>Total</b>	11216.38	199				

SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square  
F- Variance ratio

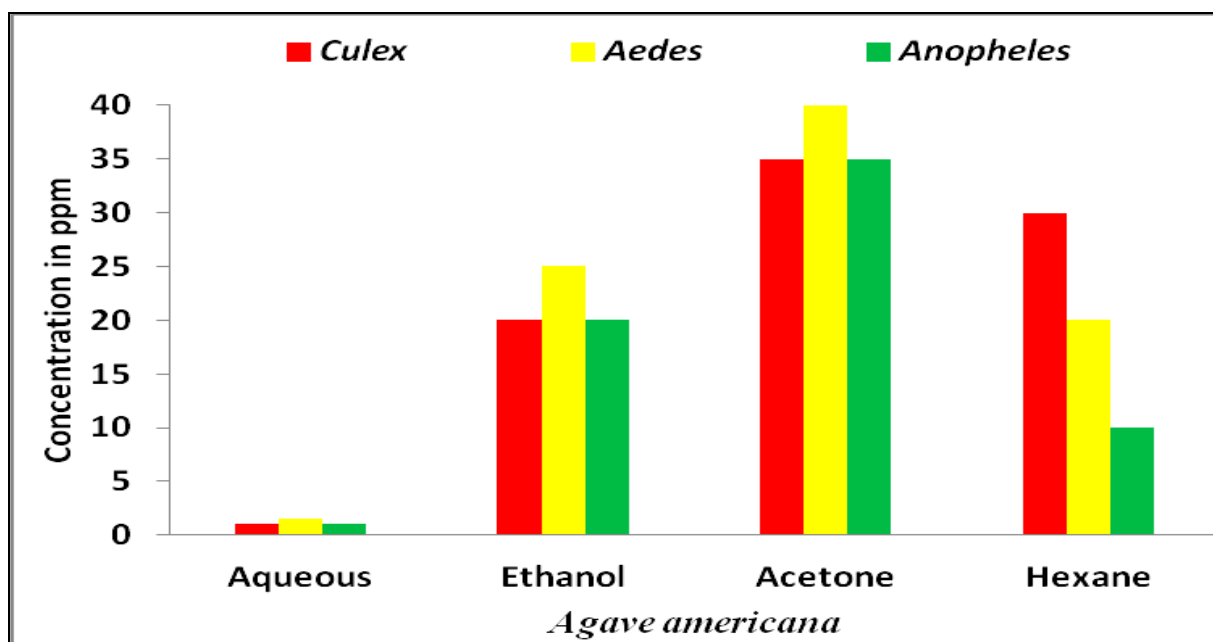
One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Acetone extract of *A. americana* showed the statistically significant effects against *An. stephensi* (p<0.05).

**Table 4.48** One way ANOVA analysis between the mortality rate of different replicates and the concentration of hexane extract of *A. americana* against *An. stephensi*.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
<b>Between Groups</b>	4382.186667	14	313.0133	1067.091	2.2E-66	1.860242
<b>Within Groups</b>	17.6	60	0.293333			
<b>Total</b>	4399.786667	74				

SS- Sum of Square                      df- degree of freedom                      MS- Mean sum of square  
F- Variance ratio

One Way ANOVA test were used between the independent variables (Concentration of extract) and dependent variables (mortality). Hexane extract of *A. americana* showed the statistically significant effects against *An. stephensi* ( $p < 0.05$ ).



**Figure 4.12** The 24hrs mortality of mosquito vectors, *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* in various concentration of different solvent extracts of *A. americana* leaves

Figure 4.12 depicted that aqueous solvent of *A. americana* shows 100% mortality against *Culex* and *Anopheles* at 1ppm, while in case of *Aedes* was found at 1.5ppm.

4.13. Quantitative analysis of ethanolic extract of *A. marmelos*

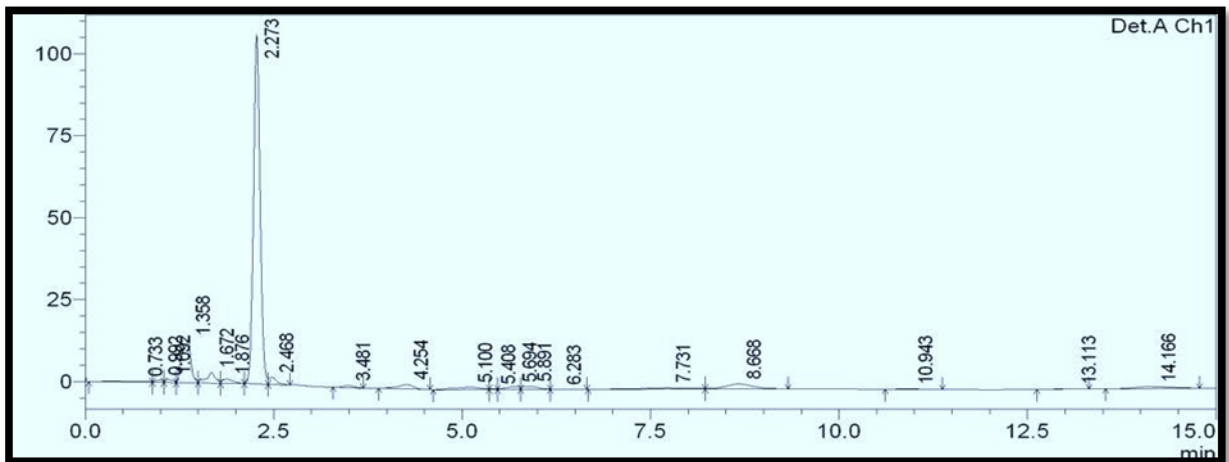


Figure 4.13 HPLC Chromatogram

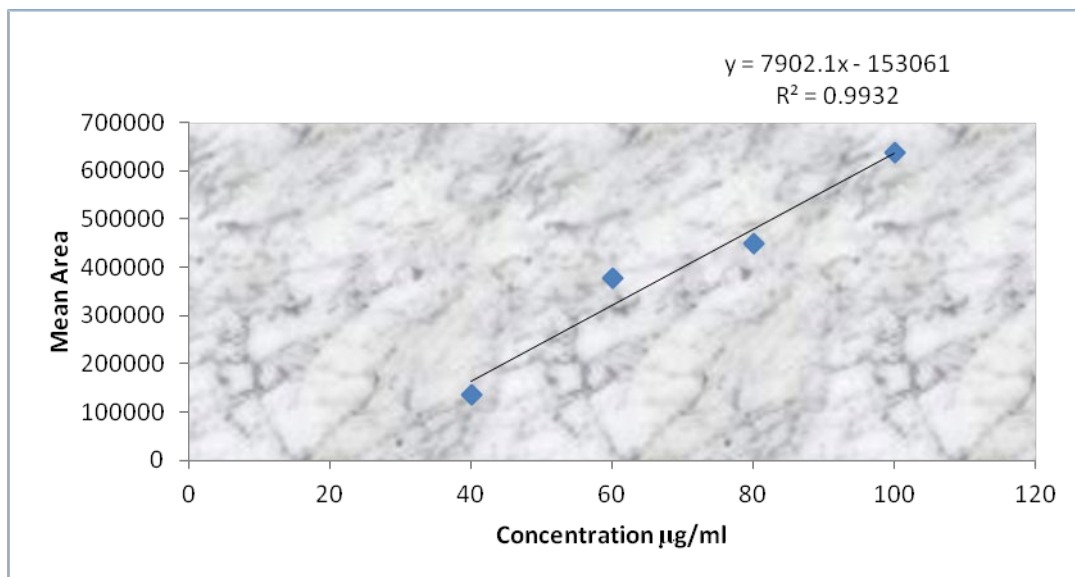
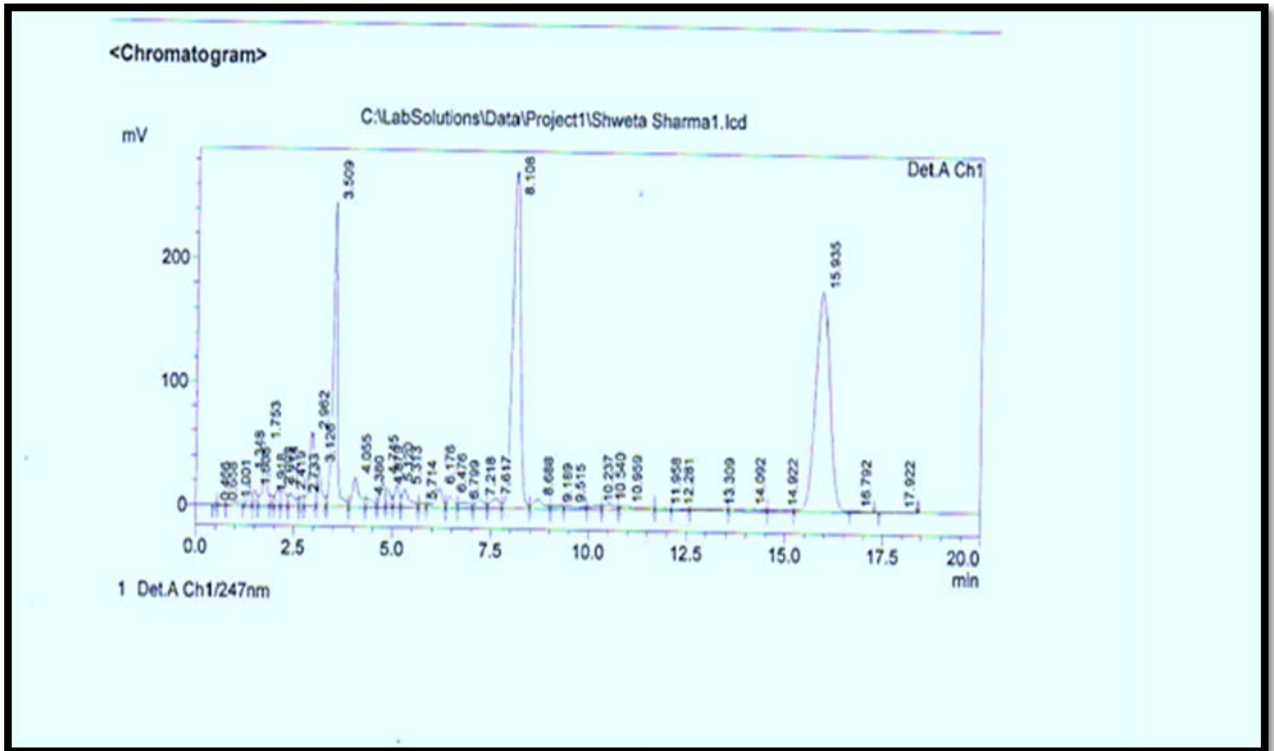


Figure 4.14 Standard graph of mermelosin with regression coefficient  $R^2 = 0.9932$ .



**Figure 4.15** Chromatogram of obtained peak of ethanol extract of *A. marmelos*



**Table 4.49** Tabular representation of HPLC chromatogram

<b>Peak</b>	<b>Ret. Time</b>	<b>Area</b>	<b>Height</b>	<b>Area %</b>	<b>Height %</b>
1	0.466	1959	317	0.014	0.029
2	0.658	5841	438	0.042	0.039
3	1.001	37639	2984	0.273	0.268
4	1.348	134504	25941	0.976	2.333
5	1.508	93848	12859	0.681	1.157
6	1.753	361848	51032	2.625	4.590
7	1.918	46146	8177	0.335	0.736
8	2.098	113863	13438	0.826	1.209
9	2.214	124023	13863	0.900	1.247
10	2.419	121060	9953	0.878	0.895
11	2.733	55866	7897	0.405	0.710
12	2.962	466533	59596	3.384	5.361
13	3.126	247831	32265	1.798	2.902
14	3.509	1845100	246291	13.384	22.154
15	4.055	288139	23934	2.090	2.153
16	4.380	89174	6754	0.647	0.608
17	4.745	193420	23468	1.403	2.111
18	4.879	120265	15104	0.872	1.359
19	5.120	157475	17644	1.142	1.587
20	5.313	185184	14979	1.343	1.347
21	5.714	35834	3295	0.260	0.296
22	6.176	195584	15780	1.419	1.419
23	6.476	111935	9394	0.812	0.845
24	6.799	79514	4094	0.577	0.368
25	7.218	101331	6964	0.735	0.626
26	7.617	122201	7970	0.886	0.717
27	8.108	3505925	272933	25.431	24.551
28	8.688	135765	7402	0.985	0.666
29	9.189	42353	2264	0.307	0.204

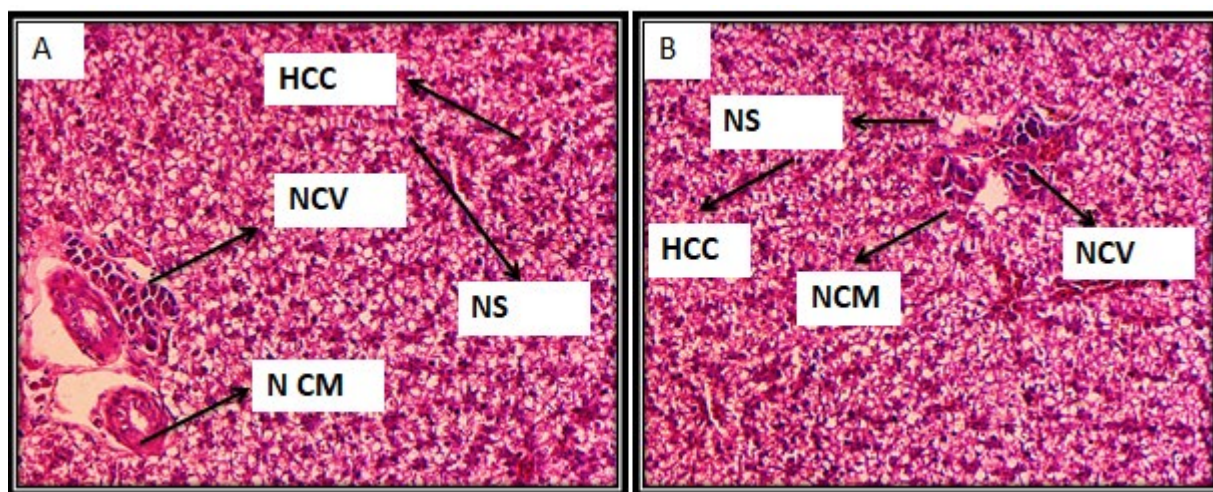
30	9.515	60628	1969	0.440	0.177
31	10.237	48439	2628	0.351	0.236
32	10.540	80546	4226	0.584	0.380
33	10.959	74652	2428	0.542	0.218
34	11.958	23697	1023	0.172	0.092
35	12.281	24223	955	0.176	0.086
36	13.309	50952	963	0.370	0.087
37	14.092	65700	1802	0.477	0.162
38	14.922	33361	1074	0.242	0.097
39	15.935	4291056	177107	31.126	15.931
40	16.792	2865	118	0.021	0.011
41	17.922	9824	391	0.071	0.035
Total		13786154	1111716	100.000	100.000

Among the all peaks, peak number 9 (see in table) has retention time 2.2 that have similarity with RT value of obtained peak in standard. Since, it depicted that present plant extract has mermelosin compound. After putting the area of respective peak (peak no 9) in obtained formula from standard graph at place Y, It was determined that plant extract has 35 µg/ml mermelosin.

Apart from this, Marmelosin compound was analyzed in ethanolic extract of *A. marmelos*. Marmelosin was detected in *A. marmelos* plant through High Performance Liquid Chromatography. This result is also supported by Sindhe *et al.*, (2014) in which Marmelosin was identified in *A. marmelos*. The relative concentrations of above phytoconstituent was determined in *A. marmelos* ethanolic extract. The method was found to give compact peaks for marmelosin (Rt of 2.2 min) and were linear 2 µg ml<sup>-1</sup> (R<sup>2</sup> = 00.9932). The findings were supported with Sindhe *et al.*, (2014).

In a research done by Wang *et al.*, (2012) Marmelosin (LC<sub>50</sub> = 3.14 and 2.88 mg L<sup>-1</sup>) was found more toxic against *Ae. aegypti* larvae respectively. The toxicity of this compound was virtually identical against larvae from the two *Culex* species, this finding indicates that the marmelosin share a mosquito repellent activity.

#### 4.16. Toxicological analysis of an ethanolic extract of *A. marmelos*



**Figure 4.16** Comparative histology of the ethanolic extract of *A. marmelos* in the liver of fish (*C. punctatus*): **A:** Control fish **B:** Treated fish

The study was performed to examine the effect of ethanolic extract of *A. marmelos* on liver of fish (*Channa punctatus*). The liver was harvested from sacrificed fish and fixed in 10% neutral buffered formalin and histopathological analysis was carried out. Results obtained from histopathology were depicted in the Figure 4.16. Results obtained from liver histopathology of treated fish were similar to liver histopathology of control fish. In both the control and treated fish liver, the normal cell membrane of epithelium, normal central vein, hepatocyte with central nuclie, and normal sinusoids were observed. Due to no significant difference between control and treated fish liver histopathology, it can be concluded that the ethanolic extract of *A. marmelos* have no toxicological properties.

This finding was corroborated by studies done in 2007 by Vinodhini *et al.*, in which, the metabolic parameters in the ethanol-intoxicated rats are significantly altered by the therapeutic administration of *A. marmelos* leaf fine powder and are effectively maintained at the normal level. These findings strongly imply that *A. marmelos* has significant hepato protective efficacy.

**Conclusion:**

In place of the traditional synthetic insecticides, plants contain a massive untapped reservoir of bioactive phytochemicals that can be widely utilised in pest control programmes. The phytoconstituents of *A. marmelos*, *A. americana* and *C. procera*, shows potential use as natural or green larvicides to combat mosquito vector. Therefore, comprehensive research should be done on the plant explored. It is beneficial to thoroughly research the larvicidal property by locating and separating its active ingredients that result in larval death, and use them in field tests in order to evaluate their entire potential instead of synthetic larvicides.



## CHAPTER 5

# SUMMARY AND CONCLUSION

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### Summary:

- The study entitled “Screening of biological larvicidal activity guided fractions of best selected available plants, against *Aedes*, *Culex* and *Anopheles* vector species of mosquitoes for herbal formulation” was undertaken in the laboratory.
- For the Computational study, the ligand molecules were retrieved from Chemspider and the inhibitory protein molecules in 3D format were obtained from PubChem database.
- To analyze the binding mechanism of ligands and target proteins, molecular docking has been performed. The activity of compounds were predicted using CB dock tool online server.
- On the basis of interaction energy score three best plants *C. procera*, *A. marmelos* and *A. americana* were selected for the further laboratory investigations.
- In the present laboratory study *C. procera*, *A. marmelos* and *A. americana* leaf extracts were obtained using various solvents such as water, ethanol, acetone and hexane according to increasing order of polarity.
- The extracts of the selected three plants (*C. procera*, *A. marmelos* and *A. americana*) were studied for its larvicidal effects against the late third instar larvae of three mosquito vector species such as dengue vector *Ae aegypti*, vector of filariasis *Cx. quinquefasciatus* and malarial vector *An. stephensi*.
- The mosquito larvae were reared at National Centre for Disease Control (NCDC, Delhi) laboratory using standard protocol.
- The leaf of selected plants were collected, shaded dried, powdered and were subjected by soxhlet extraction using water, ethanol, acetone and hexane solvents to find out their larvicidal activities.
- The tests were done according to WHO guidelines (2005). Larval mortality was

assessed after 24 hrs of bioassay.

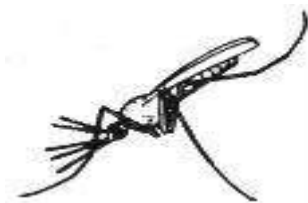
- The experimental data revealed that the ethanol extract *A. marmelos* plant showed the statistically significant larvicidal activity in comparison of other solvents (water, acetone and hexane) against the all three mosquito vectors.
- The High Performance Liquid Chromatography of *A. marmelos* ethanol extract plant confirmed the presence of marmelosin component in the extract.
- From the data it was pertinent to note that marmelosin was responsible for elicit the larvicidal activities against the *Aedes*, *Culex* and *Anopheles* vector mosquitoes.
- Toxicological analysis of ethanolic extract of *A. marmelos* was performed on fish (*Channa punctatus*) liver.
- In both the control and treated fish liver, the normal cell membrane of epithelium, normal central vein, hepatocyte with central nuclie, and normal sinusoids were assessed.
- Significantly no differences were found between the control and treated fish liver histopathology.

**Conclusion:**

Mosquitoes are responsible to transmit fatal and dangerous diseases like dengue, malaria and filariasis. In this content, the use of botanicals are an important component which should be involved on priority under Integrated Vector Management (IVM) Programmes. It has been concluded from the findings of present investigations that ethanol leaves extract of *A. marmelos* at 1.5 ppm, 1.0 ppm and 1.5 ppm can be considered as the bio-larvicides to control the larvae of *Aedes*, *Anopheles* and *Culex* mosquito vectors, respectively. It is useful in the current time wherein synthetic pesticides are developing resistance. The docking studies predicted the toxicity from physical characteristics of the secondary metabolites. Thus the hypothesis proposed in the present study is accepted since the ethanol extract of *A. marmelos* showed the remarkable larvicidal properties against the *Aedes*, *Culex* and *Anopheles* mosquito vectors. Only a few *A. marmelos* derived products have been commercialised. As a result, there is an urgent need for intensive research on this plant in order to exploit it for the control of mosquito vectors in the interest of human health around the world.

**Scope for further research:**

- Various dilutions should be used to prepare the commercial formulations.
- Many different plants could be included in this investigation to control mosquito vectors and screening their beneficial effects for the eco friendly environment.
- Plants can be identified for their active components that cause antimicrobial inhibition, and further analysis can be performed to study their structure.



## CHAPTER 6 BIBLIOGRAPHY

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- Abdalla, M. E., Khitma, H. E., & Faysal, S. Ali. (2009). Efficacy of leaves extract of *Calotropis procera* Ait. (Asclepiadaceae) in controlling *Anopheles arabiensis* and *Culex quinquefasciatus* mosquitoes. *Saudi Journal of Biological Science*, 16(2), 95–100.
- Abd, F., El, A., Abd, E. R., & Rehab, F. M. A. (2013). Proximate compositions, phytochemical constituents, antioxidant activities and phenolic contents of seed and leaves extracts of Egyptian leek (*Allium ampeloprasum* var. kurrat). *European Journal of Chemistry*, 4(3), 185-190.
- Ali, N., & El-Rabaa, F. (2010). Larvicidal activity of some plant extracts to larvae of the mosquito *Culex quinquefasciatus* (Say 1823). *European Review for Medical and Pharmacological Sciences*, 925-933.
- Argal, A., & Pathak, A. K. (2006). CNS activity of *Calotropis gigantea* roots. *Journal of Ethnopharmacology*, 106, 142-145.
- Ahmed, A. H., & El-Hamshary, E. M. (2005). Larvicidal, miracidiacidal and cercaricidal activities of the Egyptian plant, *Iris pseudacorus*. *Journal of Egyptian Society Parasitology*, 35, 41-48.
- Arivoli, S., & Tennyson, S. (2013). Screening of plant extracts for oviposition activity against *Spodoptera litura* (Fab). (Lepidoptera: Noctuidae). *International Journal of Fauna and Biological Studies*, 1(1), 20-24.
- Arunachalam, V. & Murugan, M. (2009). Larvicidal and smoke repellency effect of *Toddalia asiatica* and *Aegle marmelos* against the dengue vector, *Aedes aegypti* (Insecta: Diptera: Culicidae). *Entomological Research*, 39(1), 61–65.
- Babu, R. & Murugan, K. (1998). Interactive effect of neem seed kernel and neem gum extracts on the control of *Culex quinquefasciatus* say. *Neem Newsletter*, 15(2), 9–11.
- Baliga, S., Jimmy, R., Thilakchand, K. R., & Palatty, P. (2013). *Ocimum sanctum* (Holy Basil or Tulsi) and its phytochemicals in the prevention and treatment of cancer. *Nutrition and Cancer*, 65, 26-35.
- Bansal, S. K. S., Sharma, S., & Sherwani, M. R. (2012). Laboratory observations on the



larvicidal efficacy of three plant species against mosquito vectors of malaria, dengue/dengue hemorrhagic fever (DF/DHF) and lymphatic filariasis in the semi-arid desert. *Journal of Environmental Biology*, 33, 617–621.

Begum, N., Sharma, B., & Pandey, R. S. (2010). Evaluation of insecticidal efficacy of *Calotropis procera* and *Annona squamosa* ethanol extracts against *Musca domestica*. *Journal of Biofertilizers & Biopesticides*, 1, 1-6.

Borkent, A., & Grimaldi, D. A. (2004). The earliest fossil mosquito (Diptera : Culicidae), in Mid-Cretaceous burmese amber. *Annals of the Entomological Society of America*. 97, 882-888.

Brown, A. W. A. (1986). Insecticide resistance in mosquitoes: a pragmatic review. *Journal of the American Mosquito Control Association*, 2, 123–140.

Brahmi, F., Khodir, M., Mohamed, C., & Pierre, D. (2017). Chemical composition and biological activities of *Mentha* species. Aromatic and medicinal plants-back to nature. *London: InTech*, 47–80.

Bhatt, R. P. & Khanal, S. N. (2009). Environmental impact assessment system in Nepal - an overview of policy, legal instruments and process. *Journal of Kathmandu University for Science, Engineering and Technology*, 5,160–170.

Butt, A., Butt, B. Z., & Vehra, S. E. (2016). Larvicidal potential of *Calotropis procera* against *Aedes aegypti*. *International Journal of Mosquito Research*, 3(5), 47-51.

Campbell, F. L., Sullivan, W.W., & Smith, L.N. (1933). The relative toxicity of nicotine, anabasine, methyl anabasine and lupinine for culicine mosquito, larvae. *Journal of Economic Entomology*, 26, 500-509.

Casida, J. E., & Quistad, G. B. (1998). Golden age of insecticide research: past, present or future? *Annual Review of Entomology*, 43, 1-16.

Cetin, H., Erler, F., & Yanikoglu, A. (2004). Larvicidal activity of a botanical natural product, AkseBio2, against *Culex pipiens*. *Fitoterapia*, 75, 724-728.

Chaubey, M. K. (2017). Study of insecticidal properties of garlic, *Allium sativum* (Alliaceae) and bel, *Aegle marmelos* (Rutaceae) essential oils against *Sitophilus zeamais*. (Coleoptera: Curculionidae). *Journal of Entomology*, 14(5), 191-198.

Chellappandian, M., Sengottayan, S. N., Sengodan, K., Prabhakaran, V. S., Kandaswamy, K., Wayne, B. H., Ajmal, M. A., Chockalingam, V., Mohamed, S. E., & Dunia, A. A. F. (2022). Larvicidal and repellent activity of N-methyl-1-adamantylamine and oleic acid a major derivative of bael tree ethanol leaf extracts against dengue mosquito vector and their

- biosafety on natural predator. *Environmental Science Pollution Research International*, 29(11), 15654-15663.
- Chitme, H. R., Chandra, R., & Kaushik, S. (2005). Evaluation of antipyretic activity of *Calotropis gigantea* (Asclepiadaceae) in experimental animals. *Phytotherapy Research*, 19, 454-456.
- CSIR. (1992). The wealth of India, raw materials. Publications and Information Directorate, CSIR, New Delhi, 78(1).
- Cui, F., Raymond, M., & Qiao, C. L. (2006). Insecticide resistance in vector mosquitoes in China. *Pest Management Science*, 62(11), 1013-1022.
- Das, K., Tiwari, R. K. S., & Shrivastava, D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*, 4(2), 104-111.
- Dass, K., Sujitha, S., & Mariappan, P. (2022). Larvicidal activity of selected medicinal plants against dengue vector *Aedes aegypti*. *International Journal of Mosquito Research*, 9(1), 110-113.
- Devi, N., & Maji, T. K. (2010). Genipin Crosslinked Chitosan- $\kappa$ -carrageenan polyelectrolyte nanocapsules for the controlled delivery of isoniazid. *International Journal of Polymeric Materials*. 59, 828–841.
- Dewan, S., Sangraula, H., & Kumar, V. L. (2000). Preliminary studies on the analgesic activity of latex of *Calotropis procera*. *Journal of Ethnopharmacology*, 73, 307-311.
- Dey, P., Goyary, D., Chattopadhyay, P., Kishor, S., Karmakar, S., & Verma, A. (2020). Evaluation of larvicidal activity of *Piper longum* leaf against the dengue vector, *Aedes aegypti*, malarial vector, *Anopheles stephensi* and filariasis vector, *Culex quinquefasciatus*. *South African Journal of Botany*, 132, 482-90.
- Dharmshaktu, N. S., Prabhakaran, P. K., & Menon, P. K. (1987). Laboratory study on the mosquito larvicidal properties of leaf and seed extract of the plant *Agave americana*. *Journal of Tropical Medicine and Hygiene*, 90(2), 79-82.
- Dharmshaktu, N. S., & Menon, P. K. (1983). Larvicidal property of three species of genus agave (family: Amaryllidaceae). *Journal of Communicable Diseases*, 15(2), 135-137.
- El, A. & AL, S. (2019). Chemical constituents and pharmacological activities of *Lantana camara* – a review. *Asian Journal of Pharmaceutical and Clinical Research*, 12(12).
- Elango, G., Rahuman, A. A., Bagavan, A., Kamaraj, C., Zahir, A. A., & Venkatesan, C.

- (2009). Laboratory study on larvicidal activity of indigenous plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus*. *Parasitology Research*, *104*(6), 1381-1388.
- Elango, G., Rahuman, A. A., Zahir, A. A., Kamaraj, C., Bagavan, A., Rajakumar, G., Jayaseelan, C., Santhoshkumar T., & Marimuthu, S. (2010). Evaluation of repellent properties of botanical extracts against *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). *Parasitology Research*, *107* (3), 577-584.
- Elango, G., Rahuman, A. A., Bagavan, A., Kamaraj, C., Zahir, A. A., Rajakumar, G., Marimuthu, S., & Santhoshkumar, T. (2010). Efficacy of botanical extracts against Japanese Encephalitis vector, *Culex tritaeniorhynchus* Giles. *Parasitology Research*, *106*(2), 481-492.
- Elango, G., Rahuman, A. A., Kamaraj, C., Bagavan, A., & Zahir, A. A. (2011). Efficacy of medicinal plant extracts against malarial vector, *Anopheles subpictus* Grassi. *Parasitology Research*, *108*(6), 1437-1445.
- Elsohly, M. A., & Slade, D. (2005). Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life Science*, *78*(5), 539–548.
- Elsohly, M. A., & Gul, W. (2014). Constituents of *Cannabis sativa*. *Handbook of Cannabis*, 1-20.
- Fabiola, C. N., Jacqueline, A. L., Louise, H. G. O., Patrícia, A. P. S. S., Márcio, C. M., João, P. S. M., Sandra, R. M., & Valdir, A. B. (2015). The larvicidal activity of *Agave sisalana* against L4 larvae of *Aedes aegypti* is mediated by internal necrosis and inhibition of nitric oxide production. *Parasitology Research*, *114*(2), 543-549.
- Finney, P. J. (1971). Probit Analysis. 3rd Edition. Cambridge University Press. Cambridge, UK.
- Freitas, C. D., Viana, C. A., Vasconcelos, I. M., Moreno, F. B. B., LimaFilho, J. V., Oliveira, H. D. (2016). First insights into the diversity and functional properties of chitinase of the latex of *Calotropis procera*. *Plant Physiology Biochemistry*. *108*, 361-371.
- Funmilayo, G. F., Francis, B. A., Oluyemi, J. O., & Oluwatosin, R. O. (2020). Larvicidal activity of some plants extracts and their partitioned fractions against *Culex quinquefasciatus*. *International Journal of Tropical Disease & Health*, *44*(11), 23-34.
- Govindarajan, M., Mathivanan, T., Elumalai, K., Krishnappa, K., & Anandan, A. (2011). Mosquito larvicidal, ovicidal, and repellent properties of botanical extracts against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research*, *109*(2), 353-367.
- Ghareeb, M. A., Mohamed R. H., Hanan S. M., & Mohamed S. A. (2018). Phytochemical

analysis of *Eucalyptus camaldulensis* leaves extracts and testing its antimicrobial and schistosomicidal activities. *Bulletin of the National Research Centre*, 42, 16.

Ghosh, A., Chowdhury, N., & Chandra, G. (2012). Plant extracts as potential mosquito larvicides. *Indian Journal of Medical Research*, 135, 581-598.

Girdhar, G., Deval, K., Mittal, P. K., & Vasudevan, P. (1984). Mosquito control by *Calotropis latex*. *Pesticides*, 18, 26-29.

Goyal, M., & Mathur, R. (2011). Antimicrobial potential and phytochemical analysis of plant extracts of *Calotropis procera*. *International Journal of Drug Discovery And Herbal Research*, 138-143.

Grotenhermen, F., & Russo, E. (2002). Cannabis and Cannabinoids: Pharmacology, Toxicology, and Therapeutic Potential; Haworth Press: Binghamton, NY.

Guleria, S., & Kumar, A. (2009). Antifungal activity of *Agave americana* leaf extract against *Alternaria brassicae*, causal agent of *Alternaria* blight of Indian mustard (*Brassica juncea*). *Archives of Phytopathology and Plant Protection*, 42(4), 370-375.

Hiremath, G. I., Ahn, Y., & Kin, S. (1997). Insecticidal activity of Indian plant extracts against *Nilaparvata lugens* (Homoptera: Delphacidae). *Applied Entomology and Zoology*, 32 (1), 159-166.

Houghton, P. J., Ren, Y., & Howes, M. J. (2006). Acetylcholinesterase inhibitors from plants and fungi. *Natural Product Reports*, 23(2), 181–199.

Ibrahim, M. H., Martins, B. O., Aigbavboa, M. O., Gwaram, M. Y., Barau, B., & Garba. (2017). Crude acquired extracts of *Calotropis procera* and *Khaya senegalensis* in the management of stored cowpea weevil (*Callosobruchus maculatus*). *International Journal of Agriculture And Earth Science*, 3(4), 52-59.

Isman, M. B. (2000). Plant essential oils for pest and disease management. *Crop Protection*, 19, 603-608.

Jazem, A., Mahyoub, A., Mehmadi, R. M., Amal, H. A., Aziz, T., & Salman A. A. (2014). The effect of some plant extracts on mosquito *Aedes aegypti*. *Biosciences Biotechnology Research Asia*, 11(3), 1-9.

Jayanthi, P., Lalitha, P., & Shubashini, S. (2011). Phytochemical investigation of the extracts and the solvent fractionates of the aqueous extract of *Eichhornia crassipes*. *Journal of Pharmacy Research*, 4, 1405-1406.

Kahramano, I., Chen, C., Chen, J., & Wan, C. (2019). Chemical constituents, antimicrobial activity, and food preservative characteristics of *Aloe vera* Gel. *Agronomy*, 9, 831.

- Kakiuchi, N., Senaratne, L. R. E., Huang, S. L., Yang, X. W., Hattori, M., Pilapitiya, U., & Namba, T. (1991). Effects of constituents of Bel (*Aegle marmelos*) on spontaneous beating and calcium-paradox of myocardial cells. *Plant Medica*, 57, 43-46.
- Kaliyamoorthy, D., & Pitchaimuthu, M. (2014). Larvicidal activity of *Aegle marmelos*, *Coleus aromaticus* and *Vitex negundo* leaf extract against filarial vector *Cx. quinquefasciatus*. *Turkish Journal of Agricultural and Natural Sciences*, 1, 858-862.
- Kamana, G., Janmajoy, B., Amit, K. G., & Prasanna, D. (2014). Phytochemical constituents and pharmacological uses of medicinal plant *Achyranthes aspera*: a review. *World Journal of Pharmaceutical Research*. 4(1), 470-489.
- Kausik, B., Mondal, S., & Suresh, P. (2019). An Eye-Catching Review of *Aegle marmelos* L. (Golden Apple). *Pharmacognosy Journal*, 11(2), 207-224.
- Konno, K. (2011). Plant latex and other exudates as plant defense systems: roles of various defense chemicals and proteins contained therein. *Phytochemistry*, 72(13), 1510-1530.
- Kovendan, K., Murugan, K., & Vincent, S. (2012). Evaluation of larvicidal activity of *Acalypha alnifolia* Klein ex Wild. (Euphorbiaceae) leaf extract against the malaria vector, *Anopheles stephensi*, dengue vector, *Aedes aegypti* and Bancroftian filariasis vector, *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research*, 110, 571–581.
- Kritikar, K. R., Basu, B. D. (1999). 2<sup>nd</sup> ed. In: Indian Medicinal Plants. *International Book Distributors, Dehradun, India*, 3, 1610.
- Kumar, G., Loganathan, K., Kokati, V. B. R., Arivarasan, V. K., Chidambaram, J., & Abdul, R. (2012). Phytochemical composition, mosquito larvicidal, ovicidal and repellent activity of *Calotropis procera* against *Culex tritaeniorhynchus* and *Culex gelidus*. *Bangladesh Journal of Pharmacology*, 7, 63-69.
- Kumar, R., Ashok, K., Chandra, S. P., Nawal, K. D., & Raju, S. (2008). Insecticidal activity *Aegle marmelos* (L.) correa essential oil against four stored grain insect pests. *Internet Journal of Food Safety*, 10, 39-49.
- Kumari, R., Joshi, P. (2012). A review of Japanese encephalitis in Uttar Pradesh, India. *WHO South-East Asia Journal of Public Health*, 1(4), 374-395.
- Kumar, D., Ranjan, A., Chauhan, A., Prakash, D., & Jindal, T. (2022). Insecticidal activities of different extracts of *Calotropis procera*. In book: *New Frontiers in Environmental Toxicology*. Springer, *Cham Publication*, 91-102.
- Lahare, R. P., Yadav, H. S., Dashahre, A. K., & Bisen, Y. K. (2020). An updated review on phytochemical and pharmacological properties of *Catharanthus rosea*. *Saudi Journal of*

*Medical and Pharmaceutical Sciences*, 6(12), 759-766.

Laphookhieo, S., Phungpanya, C., Tantapakul, C., Techa, S., Tha, S., & Narmdorkmai, W. (2011). Chemical constituents from *Aegle marmelos*. *Journal of the Brazilian Chemical Society*, 22(1), 176-8.

Larhsini, M., Lazrek, H. B., Bousaid, M., Jana, M., & Amarouch, H. (1997). Evaluation of antifungal and molluscicidal properties of extracts of *Calotropis procera*. *Fitoterapia*, 68, 371-373.

Luz, S. N., Jesus, O. V., & Elena, S. (2010). Repellent activity of essential oils: A Review. *Bio Resource Technology*, 101, 372– 378.

Maazoun, A. M., Soumaya, H. H., Feten, B., Jouda, M. B. J., Chokri M., & Mohamed, N. M. (2019). Phytochemical profile and insecticidal activity of *Agave americana* leaf extract towards *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). *Environmental Science and Pollution Research*, 26(19), 19468-19480.

Marcio, V. R., Glais, D. P., Cleverson, D. T., Nadia, A. P. N., Nylane, M. N. A., Petronio, A. S., & Ana, F. U. C. (2006). Latex constituents from *Calotropis procera* display toxicity upon egg hatching and larvae of *Aedes aegypti* (Linn.). *Memorias Do Instituto Oswaldo Cruz*, 101 (5), 503-510.

Markouk, M., Bekkouche, K., Larhsini, M., Bousaid, M., Lazrek, H. B. (2000). Evaluation of some Moroccan medical plant extracts for larvicidal activity. *Journal of Ethnopharmacology*, 73, 293–297.

Mazen, A. M., Sulaibi, A. L., Carolin, T., & Thies, T. (2020). Chemical constituents and uses of *Calotropis procera* and *Calotropis gigantea* – A Review (Part I – The plants as material and energy resources). *Open Chemistry Journal*, 7, 1-15.

Menezes, B. D., Gabler, S., Greiner, R. (2015). Performance of seven commercial phytases in an in vitro simulation of poultry digestive tract. *Journal of Agriculture and Food Chemistry*, 63(27), 6142-6149.

Miller, B. R., Crabtree, M. B., & Savage, H. M. (1997). Phylogenetic relationships of the Culicomorpha inferred from 18S and 5.8S ribosomal DNA sequences (Diptera: Nematocera). *Insect Molecular Biology*, 6(2), 105-114.

Mishra, B. B., & Tripathi, S. P. (2011). Repellent activity of plant derived essential oils against *Sitophilous oryzae* (Linnaeus) and *Tribolium castaneum* (Herbst). *Singapore Journal of Scientific Research*, 1, 173-178.

Mishra, K. (2017). *Calotropis procera* : Larvicidal effect against *Culex quinquefasciatus*.

*Journal of Emerging Technologies and Innovative Research*, 4(9), 685-689.

Mishra, B. B., Tripathi, S. P., & Tripathi, C. P. M. (2016). Investigation of natural plant *Aegle marmelos* essential oil bioactivity on development and toxicity of *Tribolium castaneum* (Coleoptera: Tenebrionidae). *International Journal of Zoological Research*, 12, 40-46.

Mithilesh, K., Kurchi, B., Kuldeep, G., Ujjwal, B., Parik, K., Lalita, G., & Sanjeev, K. (2016). Identification of the temperature induced larvicidal efficacy of *Agave angustifolia* against *Aedes*, *Culex*, and *Anopheles* larvae. *Frontiers in Public Health*, 12, 3-286.

Modo, M., Bakwo, F. E. M., Tatsimo, N. S. J., & Tamesse, J. L. (2021). Evaluation of the insecticidal activity of the methanol extracts of *Calotropis procera* (Asclepiadaceae) and *Albizia lebbek* (Mimosaceae) on larvae of *Culex quinquefasciatus* Say, 1823. *Journal of Basic and Applied Zoology*, 82, 64.

Mosihuzzaman, M., & Choudhary, M. I. (2008). Protocols on Safety, efficacy, standardization, and documentation of herbal medicine. *Pure and Applied Chemistry*, 80(10), 2195–2230.

Moursy, L. E. (1997). Insecticidal activity of *Calotropis procera* extracts on the flesh fly *Sarcophaga haemorrhoidalis* Fallen. *Journal of the Egyptian Society of Parasitology*, 2, 505-514.

Moyes, C. L., Vontas, J., Martins, A. J., Ng, L. C., Koou, S. Y., Dusfour, I., Raghavendra, K., Pinto, J., Corbel, V., David, J. P., & Weetman, D. (2017). Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. *Plos Neglected Tropical Diseases*, 15(1), 1-20.

Mueen, A. K. K., Rana, A. C., & Dixit, V. K. (2005). *Calotropis* species (Asclepiadiaceae) a comprehensive review. *Pharmacognosy Magazine*, 1(2), 48-52.

Nenaah, G. E. (2013). Potential of using flavonoids, latex and extracts from *Calotropis procera* (Ait.) as grain protectants against two coleopteran pests of stored rice. *Industrial Crops and Products*, 45, 327–334.

Nivedita, G., Srivastava, S., Jain, A. & Chaturvedi, U.C. (2012). Dengue in India. *Indian Journal of Medical Research*, 373-390.

NVBDCP. (2022). <https://nvbdcp.gov.in>.

Olawale, H. O., Leonard, A., & Emmanuel, N. (2012). *Potential larvicides in Nigerian herbal recipes*, 3(10), 3783-3787.

Parrotta, J. A. (2001). *Healing plants of peninsular India*. CABI publishing.

- Patil, S. V., Patil, C. D., Salunkhe, R. B., & Salunke, B. K. (2010). Larvicidal activities of six plants extracts against two mosquito species, *Aedes aegypti* and *Anopheles stephensi*. *Tropical Biomedicine*, 27(3), 360-5.
- Patil, C. D., Patil, S. V., Salunke, B. K., Salunkhe, R. B. (2011). Bioefficacy of *Plumbago zeylanica* (Plumbaginaceae) and *Cestrum nocturnum* (Solanaceae) plant extracts against *Aedes aegypti* (Diptera: Culicidae) and nontarget fish *Poecilia reticulata*. *Parasitology Research*, 108(5), 1253–1263.
- Pawlowski, J., Szadziewski, R., Kmiecik, D., Fahrni, J., & Bittar, G. (1996). Phylogeny of the infra order Culicomorpha (Diptera: Nematocera) based on 28S RNA gene sequences. *Systematic Entomology*, 21(2), 167-178.
- Panditrao, S. S. (2020). Development of RP-HPLC method for standardization of *Aegle marmelos* (L.). *World Journal of Advanced Research and Reviews*, 7(1), 129-32.
- Piplani, M., Bhagwat, D. P., Singhvi, G., Sankaranarayanan, M., Fouce, R. B., Vats, T., & Chander, S. (2019). Plant based larvicidal agents: An overview from 2000 to 2018. *Experimental Parasitology*, 199, 92-103.
- Poinar, G. O., Zavortink, T. J., Pike, T., & Johnston, P. A. (2000). *Paleoculicis minutus* (Diptera: Culicidae) N. Gen., N. Sp., from cretaceous canadian amber, with a Summary of described fossil mosquitoes. *Acta Geologica Hispanic*, 35, 119-128.
- Poupardin, R., Reynaud, S., Strode, C., Ranson, H., Vontas, J., & David, J. P. (2008). Cross induction of detoxification genes by environmental xenobiotics and insecticides in the mosquito *Aedes aegypti*: impact on larval tolerance to chemical insecticides. *Insect Biochemistry and Molecular Biology*, 38, 540-551.
- Priestley, C. M., Williamson, E. M., Wafford, K. A., & Satelle, D. B. (2003). Thymol, a constituent of thyme essential oils, is a positive modulator of human GABA and a homo-oligomeric GABA receptor from *Drosophila melanogaster*. *British Journal of Clinical Pharmacology*, 140, 1363–1372.
- Rahuman, A. A., Gopalakrishnan, G., Venkatesan, P., & Geetha, K. (2008). Larvicidal activity of some Euphorbiaceae plant extracts against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera:Culicidae). *Parasitology Research*, 102, 867-873.
- Ramya, S., & Jayakumararaj, R. (2009). Antifeedant activity of selected ethno-botanicals used by tribals of vattal hills on *Helicoverpa armigera* (Hübner). *Journal of Pharmacy Research*, 2(8), 1414-1418.
- Ramos, M. V., Bandeira, G. P., Freitas, C. D. T., Nogueira, N. A. P., Alencar, N. M. N.,



- Sousa, P. A. S., Carvalho, A. F. U. (2006). Latex constituents from *Calotropis procera* (R. Br.) display toxicity upon egg hatching and larvae of *Aedes aegypti* (Linn.). *Memorias do Instituto Oswaldo Cruz*, 101(5), 503-10.
- Rana, B. K., Singh, U. P., & Taneja, V. (1997). Antifungal activity and kinetics of inhibition by essential oil isolated from leaves of *Aegle marmelos*. *Journal of Ethnopharmacology*, 57, 29-34.
- Rattan, R. S. (2010). Mechanism of action of insecticidal secondary metabolites of plant origin. *Crop Protection*, 29, 913–920.
- Rathy, M. C., Sajith, U., Harilal, C. C. (2015). Larvicidal efficacy of medicinal plant extracts against the vector mosquito *Aedes albopictus*. *International Journal of Mosquito Research*, 2(2), 80-2.
- Reed, W., Carroll, J., Agramonte, A., & Lazear, J. W. (1900). The etiology of Yellow fever—a preliminary note. *Public Health Papers And Reports*, 26, 37-53.
- Reegan, A. D., Kinsalin, A. V., Paulraj, M. G., & Ignacimuthu, S. (2013). Larvicidal, ovicidal and repellent activities of marine sponge *Cliona celata* (Grant) extracts against *Culex quinquefasciatus* Say and *Aedes aegypti* L. (Diptera: Culicidae). *ISRN Entomology*, 1–8.
- Reegan, A. D., Gandhi, M. R., Paulraj, M. G., & Ignacimuthu, S. (2015). Ovicidal and oviposition deterrent activities of medicinal plant extracts against *Aedes aegypti* L. and *Culex quinquefasciatus* Say mosquitoes (Diptera: Culicidae). *Osong Public Health And Research Perspectives*, 6(1), 64-69.
- Rejiniemon, T. S., Arasu, M. V., Duraipandiyar, V., Ponmurugan K., Dhabi N. A., Arokiyaraj, S., Agastian, P., & Choi K. C. (2014). In-vitro antimicrobial, antibiofilm, cytotoxic, antifeedant and larvicidal properties of novel quinone isolated from *Aegle marmelos* (Linn.) Correa. *Annals of Clinical Microbiology and Antimicrobials*, 13(48), 1-9.
- Renu, H. V. M., Dubey, N. K., & Dixit, S. N. (1986). Mycotoxic properties of the essential oil of *Aegle marmelos*. *Beiträge Zur Biologie Der Pflanzen*, 60, 325-332.
- Riju, S., Sudarshana, M., & Bulbuli, K. (2017). Insecticidal activities of the essential oil of *Aegle marmelos* (Linnaeus, 1800) against *Aedes aegypti* (Linnaeus, 1762) and *Culex quinquefasciatus* (Say, 1823). *Universal Journal of Agricultural Research*, 5(5), 304-311.
- Ross, R. (1898). The Role of the mosquito in the evolution of the malaria parasite. *Lancet*, 488-489.
- Ross, S., & Elsohly, M. A. (1995). Constituents of *Cannabis sativa* L. XXVIII—A review of the natural constituents: 1980–1994. *Zagazig Journal of Pharmacology Science*, 4, 1–10.

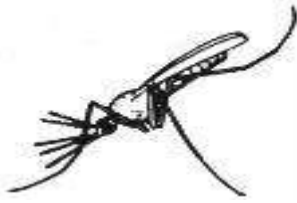
- Roark, R. C. (1947). Some promising insecticidal plants. *Economic Botany*, 1, 437–445.
- Roberts, R. J. (2001). Fish Pathology. 3<sup>rd</sup> edn. *W. B. Saunders Publishing, London, UK*.
- Russell, T. L., Kay, B. H., & Skilleter, G. A. (2009). Environmental effects of mosquito insecticides on saltmarsh invertebrate fauna. *Aquatic Biology*, 6, 77–90.
- Salunke, B. K., Kotkar, H. M., Mendki, P. S., Upasani, S. M., & Maheswari, V. L. (2005). Efficacy of flavonoids in controlling *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae), a post-harvest pest of grain legumes. *Crop Protection*, 24(10), 888-893.
- Samarasekera, J. K. R. R., Bhupinder, P. S., Khambay, & Hemalal, K. P. (2004). A new insecticidal protolimonoid from *Aegle marmelos*. *Formerly Natural Product Letters*, 18(2), 117-122.
- Samuel, T., Ravindran, K. J., & Arivoli, S. (2011). Screening of plant extracts for ovicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Elixir Applied Botany*, 40, 5456-5460.
- Santhoshkumari, K. S., & Devi, K. S. (1990). Hypoglycemic effect of a new medicinal plants. *Ancient Science of Life*, 4, 221-223.
- Selvan, P. S., Senthoorraja, R., Ramesh, V., Jebanesan, A. (2021). Field evaluation of toxicity of plant extracts against vector of filariasis *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae). *South African Journal of Botany*, 139, 58-66.
- Shalan E. A. S., Canyon, D., Younesc, M. W. F., Abdel-Wahab, H., & Mansoura, A. H. (2005). A review of botanical phytochemicals with mosquitocidal potential. *Environment International*, 31, 1149–1166.
- Shahi, M., Hanafi-Bojd, A. A., Iranshahi, M., Vatandoost, H., & Hanafi- Bojd, M. Y. (2010). Larvicidal efficacy of latex and extract of *Calotropis procera* (Gentianales: Asclepiadaceae) against *Culex quinquefasciatus* and *Anopheles stephensi* (Diptera: Culicidae). *Journal Vector Borne Disease*, 47, 185-188.
- Shaktu, N. S., & Menon, P. K. (1983). Larvicidal property of three species of genus agave (fam: Amaryllidaceae). *Journal of Communicable Diseases*, 15(2), 135-137.
- Sharma, P., Mohan, L., & Srivastava, C. N. (2006). Impact analysis of neem kernel extracts on the development profile of *Anopheles stephensi*. *Journal of Asia-Pacific Entomology*, 9, 11-17.
- Sharma, S. R., Dwivedi, S. K., Varshney, V. P., & Swarup, D. (1996). Antihypoglycemic and Insulin release effects of *Aegle marmelos* leaves in streptozotocin diabetic rats. *Phytotherapy Research*, 10, 426-428.

- Sharma, V. P. (2001). Health hazards of mosquito repellents and safe alternatives. *Current Science*, 80(3), 341-343.
- Shelke, S.P. (2020). Development of RP-HPLC method for standardization of *Aegle marmelos* (L.). *World Journal of Advanced Research and Reviews*, 7(1), 129-132.
- Shreya, N., Raghavendra, N. P., Mukherji, V., Maria, V. R., Pradeep, A. S., Ghosh, S. K., & Bindhu, O. S. (2012). Larvicidal activity of *Calotropis gigantea* (L.) on dengue and chikungunya vector *Aedes aegypti*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 118-121.
- Shinde, P. B., Katekhaye, S. D., Mulik, M. B., & Laddha, K. S. (2014). Rapid simultaneous determination of marmelosin, umbelliferone and scopoletin from *Aegle marmelos* fruit by RP-HPLC. *Journal of food science and technology*, 51(9), 2251-5.
- Singh, R. K., Mittal, P. K., & Dhiman, R. C. (2005). Laboratory study on larvicidal properties of leaf extract of *Calotropis procera* (Family-Asclepiadaceae) against mosquito larvae. *Journal of Communicable Diseases*, 37(2), 109-113.
- Singh, R. K., Mittal, P. K., Kumar, G., Dhiman, R. C. (2014). Evaluation of mosquito larvicidal efficacy of leaf extract of a cactus plant, *Agave sisalana*. *Journal of Entomology and Zoology Studies*, 2(1), 83-86.
- Singh, M., Kumari, S., Attri, R., & Kumar, S. (2015). Impact of *Calotropis procera* leaf extracts on the survival, morphology and behaviour of Dengue vector, *Aedes aegypti* L. *Journal of Undergraduate Research and Innovation*, 1(3), 96- 107.
- Singhi, M., Joshi, V., & Dam, P. K. (2006). Studies on *Calotropis procera* as larvicidal and repellent plant against vectors of dengue and DHF in Rajasthan, India. Annual Report 2005-06. Jodhpur: *Desert Medicine Research Center*, 24–28.
- Singhi, M., Purohit, A., & Chattopadhyay, S. (2015). Effectiveness and feasibility of methanol extracted latex of *Calotropis procera* as larvicide against dengue vectors of western Rajasthan, India. *Journal of Vector Borne Diseases*, 52(2), 142-146.
- Singhia, M., Joshi, V., Sharma, R. C., & Sharma, K. (2004). Ovipositioning behavior of *Aedes aegypti* in different concentrations of latex of *Calotropis procera*: studies on refractory behavior and its sustenance across genotrophic cycles. *Dengue Bulletin*, 28, 184-188.
- Singanani, V., Singanani, M., & Begum, H. (2007). The hepatoprotective effect of bael leaves (*Aegle marmelos*) in alcohol induced liver injury in albino rats. *International Journal of Science & Technology*, 2(2), 83-92.

- Singh, M., Kumari, S., Attri, R., & Kumar, S. (2015). Impact of *Calotropis procera* leaf extracts on the survival, morphology and behaviour of dengue vector, *Aedes aegypti* L. *Journal of Undergraduate Research and Innovation*, 1, 96-107.
- Sivagnaname, N., & Kalyanasundaram, M. (2004) Laboratory evaluation of methanolic extract of *Atlantia monophylla* (Family: Rutaceae) against immature stages of mosquitoes and non-target organisms. *Memorias Do Instituto Oswaldo Cruz*, 99, 115-118.
- Snehlata, K., & Sheel, R. (2020). Insecticidal Activity of Acetone extract of leaves of *Aegle marmelos* (L.) against Lepidopteran insect pest. *International Journal of All Research Education and Scientific Methods*, 8(10), 516-521.
- Soni, P., Siddiqui, A. A., Dwivedi, J., & Soni, V. (2012). Pharmacological properties of *Datura stramonium* L. as a potential medicinal tree: An overview. *Asian Pacific Journal Tropical Biomedicine*, 2(12), 1002-1008.
- Sowmyashree, K., Chalannavar, R. K., Ghosh, S. K., Nityasree, B. R., & Supriya S. (2019). Effect of essential oils of *Aegle marmelos* (l.) correa and *Psidium guajava* l. on larvae of malaria vector *Anopheles stephensi* liston. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*, 5(1), 704-711.
- Sritabutra, D., Soonwera, M., Sirirat, S., & Pongjai, S. (2011). Evaluation of herbal essential oil as repellents against *Aedes aegypti* (L.) and *Anopheles dirus* Peyton & Harrion. *Asian Pacific Journal of Tropical Biomedicine*, 1(1), 124-128.
- Staples, G., & Herbst, D.R. (2005). Tropical garden flora: *Bishop Museum Press*.
- Sukumar, K., Perich, M. J., Boobar, L. R. (1991). Botanical derivatives in mosquito control: a review. *Journal of the American Mosquito Control Association*, 7, 210–37.
- Su, T., & Mulla, M. S. (1998). Ovicidal activity of neem products (azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). *Journal of the American Mosquito Control Association*, 14(2), 204-209.
- Sunita, S. (2012). Marmelosin based standardization of ayurvedic formulations containing *Aegle marmelos* using RP-HPLC method. *Journal of Pharmacy Research*, 5(4), 2224-2227.
- Tinto, W., Boyce, J. L. S., & Reynolds, W. (2005). Constituents of *Agave americana* and *Agave barbadensis*. *Fitoterapia*. 76(6), 594-597.
- Tahir, H. M., Ishaq, T., Mukhtar, M. K., Khan, S. Y., & Ahmed, K. (2013). Potential use of *Calotropis procera* (Milk Weed) to Control *Culex quinquefasciatus* (Diptera: Culicidae). *Pakistan Journal of Zoology*, 45(3), 615-621.
- Taubes, G. A. (1997). Mosquito bites back. *New York Times Magazine*, 40-46.

- Thomas, T. G., Rao, S., & Lal, S. (2004). Mosquito larvicidal properties of essential oil of an indigenous plant, *Ipomoea cairica* Linn. *Japanese Journal of Infectious Diseases*, 57, 176-177.
- Torres, R. C., Garbo, A. G., Walde, R. Z. (2015). Characterization and bioassay for larvicidal activity of *Anacardium occidentale* (cashew) shell waste fractions against dengue vector *Aedes aegypti*. *Parasitology research*, 114(10), 3699-4702.
- Turner, C. E., Elsohly, M. A., & Boeren, E. G. (1980). Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. *Journal of Natural Products*, 43(2), 169-234.
- Udupa, S. L., Udupa, A. L., & Kulkarni, D. R. (1994). Studies on the anti-inflammatory and wound healing properties of *Moringa oleifera* and *Aegle marmelos*. *Fitoterapia*, 65,119-123.
- Uthu, B., & Kamalanathan, A., & Janarthanan, S. (2020). In silico molecular docking of kappa-carageenan against d7 salivary gland protein of adult female *Anopheles stephensi*, 9, 17-31.
- Van, Q. E., Simon, G., André, A., Dewelle, J., Yazidi, M. E., & Bruyneel, F. (2005). Identification of a Novel Cardenolide (2"-Oxovoruscharin) from *Calotropis procera* and the hemisynthesis of novel derivatives displaying potent in vitro antitumor activities and high in vivo tolerance: structure-activity relationship analyses. *Journal of Medicinal Chemistry*, 849-856.
- Veerappan, A., Miyazaki, S., & Dhananjayan, R. (2005). Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of *Aegle marmelos* Corr. *Journal of Ethnopharmacology*, 96(1-2), 159-163.
- Vogel, A. I. (1978). Textbook of practical organic chemistry. *The English Language Book Society and Longman, London*, 1368.
- Wadhvani, B. D., Mali, D., Vyas, P., Nair, R., & Khandelwal, P. (2021). A review on phytochemical constituents and pharmacological potential of *Calotropis procera*. *RSC Advances*, 11, 35854–35878.
- Wang, Z., Kim, J. R., Wang, M., Shu, S., & Ahn, Y. J. (2012). Larvicidal activity of *Cnidium monnieri* fruit coumarins and structurally related compounds against insecticide-susceptible and insecticide-resistant *Culex pipiens pallens* and *Aedes aegypti*. *Pest Management Science*, 68(7), 1041-1047.
- Wilkerson, R. C., Linton, Y. M., Fonseca, D. M., Schultz, T. R., Price, D. C., & Strickman, D. A. (2015). Making mosquito taxonomy useful: a stable classification of tribe Aedini that balances utility with current knowledge of evolutionary relationships. *PloS one*, 10(7), 1-26.

- WHO, (1996). Report of the WHO informal consultation on the evaluation and testing of insecticides. Protocols for laboratory and field evaluation of insecticides and repellents. CTD/WHOPES/IC-96,1,Geneva
- WHO, (2005). Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization communicable disease control, prevention and eradication, WHO pesticide evaluation scheme (WHO/CDS/WHOPES/GCDPP/2005.13).
- WHO, (2014). A Global brief on Vector-Borne Diseases. Geneva: World Health Organization, WHO/DCO/WHD/2014.1.
- WHO, (2015). World Malaria Report. Geneva: World Health Organization.
- WHO, (2017). World Malaria Report. Geneva: World Health Organization.
- Yadav, R. K., Nandy, B. C., Siddhartha Maity, S. M., Sarkar, S., & Saha, S. (2015). Phytochemistry, pharmacology, toxicology, and clinical trial of *Ficus racemosa*. *Pharmacognosy Review*, 9(17), 73–80.
- Yakubu, M. S., Mohammed, A. & Tanko, M. M. (2021). Lethal Effects of *Calotropis procera* leaves extract on mosquito larvae. *International Journal for Research in Applied Sciences and Biotechnology*, 4(8), 100-103.



## CHAPTER 7

# ACRONYMS AND ABBREVIATIONS

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The following symbols and abbreviations are used throughout the study:

<i>Abbreviations</i>	<i>– Referent</i>
Cx	– <i>Culex</i>
o C	– Degree Celsius
cm	– Centimeter
cm <sup>2</sup>	– Square Centimeter
DGHS	– Directorate General of Health Services
Figure.	– Figure
Hrs	– Hours
ICMR	– Indian Council of Medical Research
IARI	– Indian Agricultural Research Institute
Kg	– Kilogram
L	– Litre
LD <sub>50</sub>	– Lethal dose – 50
LC <sub>50</sub>	– Lethal Concentration – 50
Max.	– Maximum
Min.	– Minimum
Min.	– Minute (s)
mg / l	– Milligram Per Litre
mm	– Millimetre
ml	– Millilitre
mg	– Milligram
me	– Milliequivalent
MH&FE	– Ministry of Health and Family Welfare
NICD	– National Institute of Communicable Diseases
NVBDCP	– National Vector Borne Diseases Control Programme

NIMR	– National Institute of Malaria Research
nm	– Nanometre
ppm	– Part Per Million
pg/ml	– picograms per milliliter
pm	– Picometre
sec	– Second (s)
Temp.	– Temperature
vol	– Volume
WHO	– World Health Organization
wt	– Weight
µm	– Micrometre
µl	– Microlitre
&	– And

## **APPENDIX-1**



## **LIST OF PUBLICATIONS**

1. Shweta Kaushik, Neeta Raj Sharma, TG Thomas, Abhay Kumar Sharma, Anu Bansal. Indigenous Plants and their Larvicidal Potential against Indian Mosquito Vectors: A Review. *Journal of Communicable Diseases*. 2019, vol. 51, Issue 2, Pg. No. 59-72.
2. Shweta Kaushik, Neeta Raj Sharma, Shashank Garg, Anu Bansal, TG Thomas. Larvicidal effects of *Calotropis procera* leaf extracts against *Aedes aegypti*, vector of Dengue fever. *Entomon*. 2022, vol. 47, Issue 4, Pg. No. 415-420.



Review Article

# Indigenous Plants and their Larvicidal Potential against Indian Mosquito Vectors: A Review

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## A B S T R A C T

All over the world, millions of people are suffering from mosquito borne diseases spreading by bacteria, viruses or parasites and transmitted by mosquitoes to humans. It is estimated that about billions of currencies are spent by nations annually due to these diseases and millions of people die as a consequence of catching mosquito borne diseases. The World Health Organization has recorded mosquitoes borne diseases as one of the topmost threats to public health, particularly in developing countries. In India, it has been estimated that annually more than 40 million people suffer from mosquito illness. Mosquito control includes target killing the larvae of mosquitoes even before they emerge into adults via using botanical extracts as an alternative larvicides. Herbal plants having a good medicinal values and potential so now a days it has been used as an insecticide at an individual and community level. These are non-toxic and biodegradable measures that are easily available and inexpensive depicting broad spectrum potential against the various strains of mosquitoes. Existing studies have taken in account the probit analysis for the calculation of percentage,  $LC_{50}$ ,  $LC_{99}$  values and 95% confidence limits to propound the observed relationship between the mortality percentage of larvae and logarithmic concentration of the active constituents found in herbal extracts. In this article, we reviewed on the current state of knowledge available on the larvicidal value of plant extracts and mosquitocidal activity, the nature of active parts of plant and promising advances, knowledge to make herbal or biological control of various species of mosquitoes as a potential eco-friendly and safe larvicides.

**Keywords:** Mosquito Borne Disease, Larvicide, Herbal Plants, Insecticide

## Introduction

The different species of Mosquito play their role as a vector for most of the life suffering diseases namely dengue fever, malaria, chikungunya fever, yellow fever, filariasis, encephalitis and West Nile virus infection, throughout

the world. Approximately out of 4000 different species, less than 10% of mosquito species are suitable vectors of pathogenic agents of mosquito illness diseases. According to Taubes (1997) diseases transmitted due to mosquitoes are said to be a prominent cause of mislaying of human life

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## Larvicidal effects of *Calotropis procera* leaf extracts against *Aedes aegypti* (L), vector of dengue fever

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**ABSTRACT:** Leaf extracts of *Calotropis procera* were tested against late third instar larvae of *Aedes aegypti* mosquito. Soxhlet extraction of the dried leaves powder with polar and non polar solvents (water, ethanol, hexane and acetone) was carried out. Larvicidal effects of plant extracts were observed after 24h of exposure. The control group showed no mortality. Ethanolic extract was found more toxic with LC<sub>50</sub> 1.923 ppm and LC<sub>90</sub> 8.83 ppm followed by aqueous extract (LC<sub>50</sub> 2.607 ppm and LC<sub>90</sub> 11.903 ppm), acetone extract (LC<sub>50</sub> 4.1 ppm and LC<sub>90</sub> 16.471 ppm) and hexane extract (LC<sub>50</sub> 5.364 ppm and LC<sub>90</sub> 31.759 ppm). As the ethanolic extract of *C. procera* leaves showed significant larvicidal properties, it can be used as an eco-friendly alternative for the control of *Ae. aegypti* vector. © 2022 Association for Advancement of Entomology

**KEY WORDS:** Ethanolic extract, probit analysis, toxicity, biopesticide

Mosquitoes transmit a myriad of harmful diseases like dengue, malaria, chikungunya, lymphatic filariasis and Japanese encephalitis. Approximately 700 million people suffer from such mosquito borne diseases each year that gradually results in about 1 million deaths annually (Taubes, 1997). The distribution of vector borne diseases is determined by complex demographic factors including environmental and social factors as well. Annual dengue incidences are estimated to be in the order of 100 million symptomatic and 300 million asymptomatic. The greatest burden is seen in Asia (75%) followed by Latin America (14%) and Africa. India suffers from three vector-borne diseases, malaria, lymphatic filariasis and visceral leishmaniasis (WHO, 2017). *Aedes aegypti* (Diptera, Culicidae) is the main vector of dengue and chikungunya (WHO, 2022). To control the

proliferation of vector species of mosquitoes so many synthetic insecticides have been used worldwide. However, none of the formulations are promising due to its high cost, less environmental friendly, harmful effect on public health and increasing incidence of insecticide resistance. Because of these harmful effects on the public health and environment, herbal eco friendly formulations are in demand (Nerio *et al.*, 2010; Sritabutra *et al.*, 2011 and Reegan *et al.*, 2013). Further, as an alternative, the chemicals derived from the different parts of the plants can be used as a repellent, larvicide, ovipositional attractant and insect growth regulator (Babu and Murugan, 1998; Demirak and Canpolat, 2022).

*Calotropis procera* (Aiton) Dryand belongs to the family Asclepiadaceae and is mostly found in

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## **LIST OF CONFERENCES**

1. Participated in Poster presentation on Mosquito larvicidal properties of indigenous plants in the international conference on Innovative strategies for Sustainable Water Management held from 17-11-2017 to 18-11-2017 at Lovely Professional University, Punjab.
2. Participated in International Conference on Sustainability: Life on Earth 2021 (ICS-LOE 2021) held on 17-18 December 2021 at Lovely Professional University, Punjab.



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# Certificate of Participation

This is to certify that Dr./Mr./Ms. Shweta Kaushik  
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on Innovative Strategies for Sustainable Water Management held from 17-11-2017 to 18-11-2017  
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## WORKSHOP PARTICIPATION

Workshop on Quantitative Data Analysis and Statistical Design of Scientific Experiments held from 13-11-2017 to 16-11-2017 at Lovely Professional University, Punjab.

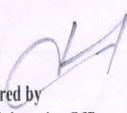
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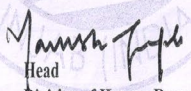
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Head  
Division of Human Resource

  
Head  
Human Resource Development Center

## PATENT PUBLISHED

Patent Published on the topic “A NOVEL ECOFRIENDLY FORMULATION FOR CONTROLLING DENGUE VECTOR” with application no.TEMP/E1/48759/2022-DEL and 28391.

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4	202211043003	TEMP/E-1/48623/2022-DEL	1600	28391	FORM 1	Full	A NOVEL PROCESS FOR ADAMANT PLASTER FROM INDUSTRIAL EFFLUENT
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