

**BIOACTIVITY GUIDED ISOLATION AND FORMULATION OF
NATURAL PESTICIDES AGAINST THREE DIFFERENT
MOSQUITO SPECIES**

Thesis submitted to

**THE KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH,
BELAGAVI**

(KLE DEEMED UNIVERSITY)

[Declared as Deemed-to-be-University u/s 3 of the UGC Act, 1956 vide

Govt. of India Notification No.F.9-19/2000-U.3 (A)]

(Accredited 'A⁺' Grade by NAAC) (3rd cycle)

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For the award of the degree of

Doctor of Philosophy

In the Faculty of

Pharmacy

by

MS. PRASHANTHI P

(Registration No: KLEU/Ph.D./2014-2015/D012140015)



**Under the Guidance of
DR. RAJAMMA AJ**

March 2025

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ACKNOWLEDGMENT

I am thankful to Dr. Rajamma AJ, Principal and Head Department of Pharmacognosy, K.L.E. University College of Pharmacy, Bengaluru, my guide, for giving excellent guidance, persistent inspiration and I shall remain indebted to her for having a premeditated belief in my quest for excellences, honesty and respected for the moral and ethics.

I am especially thankful to K.L.E. University Belagavi, for providing an excellent opportunity to carry out the dissertation work under the university.

I am taking this opportunity to thank Dr. Hippargi K.L.E.C.O.P, Bengaluru, (Former Principal), Dr. Subash S. Karki, Head Department of Pharmaceutical Chemistry for providing all the necessary facilities at the campus. I am indeed grateful for the valuable support provided by Dr. Shivakumar H.N, Prof and Head Department of Pharmaceutics; K.L.E.C.O.P, Bengaluru. I am also sincerely thankful to all teaching and non-teaching staff members, for their timely help and encouragement.

I express my gratitude to Dr. Sushanth K Ghosh, Former Scientist F & Officer-in-Charge, National Institute of Malaria Research-Field Unit, Indian Council for Medical Research (ICMR), Devanahalli, Bengaluru whose guidance was articulated. I am also grateful to the former Officer-in-Charge & Scientist Dr. Shreehari U and Lab technicians- Mr. Jaiswal, Mr. Rajan, and project Assistants, and other research fellows at the institute for their immense help & support in the research work. It is my privilege to extend my gratitude to Dr. Roopashree T.S, & Dr. Anita S, Department of Pharmacognosy, Government College of Pharmacy, Bengaluru, for their help in HPTLC. I am thankful to NMR Research Centre IISc.-Bengaluru for the NMR and Acquity Labs-Bengaluru, for the Mass spectra.

I wish to express my sincere thanks to the Director of Academic Affairs and the Vice chancellor for their help and kind support.

Thankful & bound to the almighty that I may ever remain.....

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List of abbreviations used

Abbreviation/ symbols	Meaning
-	To
<	Less than
≤	Less than or equal to
#	Sieve number
%	Percentage
/	Per
:	As
+	Plus (add)
+ ve	Present
- ve	Absent
>	Greater than
≥	Greater than or equal to
±	Plus or Minus
#	Mesh Number
µg	Microgram
µl	Microliter
µm	Micrometer
°C	Degree Celsius
AL	<i>Artocarpus lakoocha</i> Roxb
ALL	<i>Artocarpus lakoocha</i> Roxb leaves
AchE	Acetylcholine Esterase
ALF	<i>Artocarpus lakoocha</i> Roxb Fruits
avg.	Average
AZ	Azadirachtin
C1	Column number 1
C13	Carbon isotope 13
C2	Column number 2
CH	Chloroform
CHE	Chloroform Extract
Cm	Centimetre

cm ⁻¹	Centimeter inverse
Cm ²	Centimetre square
conc.	Concentration
CVA	Cleome viscosa Linn., Aerial Parts
dil.	Dilute
DMSO	Dimethyl sulfoxide
DDT	Dichlorodiphenyltrichloroethane
DEN	Dengue virus
E	Extract
EA	Ethyl acetate
ED	Effective Deterrence
ER ₅₀	Effective 50% repellence to oviposition
ER ₉₀	Effective 90 % repellence to oviposition
et. al.	And others
etc.	And other things
ET	Ethanol
ETE	Ethanol Extract
Ext.	Extraction
F	Fraction
FeCl ₃	Ferric Chloride
FLT	Floating Lag Time In Seconds
FTIR	Fourier Transform Infrared Spectroscopy
JE	Japanese Encephalitis
H ₂ SO ₄	Sulphuric Acid
HPLC	High Performance or Pressure Liquid Chromatography
HPTLC	High-Performance Thin Layer Chromatography
Hr	Hour
IGR	Insect Growth Regulator
ICMR	Indian Council For Medical Research
IE ₅₀	Inhibition To Emergence 50%
IE ₉₀	Inhibition To Emergence 90%
IGR	Insect Growth Regulator

IP	Indian Pharmacopoeia
JH	Juvenile Hormone
JHA	Juvenile Hormone Mimicking Activity
KBr	Potassium Bromide
LC ₅₀	Lethal concentration with 50% Mortality
LC ₉₀	Lethal concentration with 90% Mortality
LCL	Lower Confidence Limit
L: D	Light: Dark
Mg	Milligram
MHz	Megahertz
min	Minute
mL	Milliliter
mmol	Mili mole
Mp	Melting point
Nm	Nanometer
NMR	Nuclear Magnetic Resonance Spectroscopy
NR	Natural Remedies
OH-	Hydroxyl
$p \leq 0.05$	Level of significance
PE	Petroleum ether
PEE	Petroleum ether extract
ppm	Parts per million
PSF	Predator Safety factor
Rf	Retention factor
RH	Relative Humidity
RT	Retention Time
SD	Standard deviation
EV	Soluble extractive values
SI	Suitability index
T.S.	Transverse Section
FTs	Floating time in seconds
TFT	Total floating time

TLC	Thin Layer Chromatography
™	Trademark
UCL	Upper Confidence Limit
UV	Ultraviolet Spectroscopy
Vis	Visible
viz.,	Namely
w/w	Weight by Weight
WHO	World Health Organization
WHOPES	World Health Organization Pesticidal Evaluation Scheme
WE	Water extract
WL	White light
X ²	Chi-square
A	Alpha
λ max	Lambda max

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ABSTRACT

BACKGROUND: Mosquitoes transmit viruses and parasites, which increase human mortality. Various methods are being adopted to control mosquitoes involving the use of synthetic pesticides with unexpected consequences on both target and non-target organisms. As substitutes for synthetics, natural products with agronomic qualities of biodegradability, low resistance, and those preserving ecological equilibrium are to be preferred.

OBJECTIVE: To identify active extracts, isolate pesticidal compounds, and develop sustainable eco-friendly formulations from the extracts of *Cleome viscosa* Linn. and or *Artocarpus lakoocha* Roxb.

METHODOLOGY: Three target species- *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* were subjected to larvicidal activity, oviposition deterrence, and IGR-Juvenile hormone-mimicking studies were performed as per WHOPEs. Bioactivity-guided isolation of larvicidal compounds was carried out and isolated compounds were tested for larvicidal activity and safety on larvivorous fish *Poecilia reticulata*. Larvicidal formulations as floating tablets, liquid concentrates and wettable powders with benefits of integration and release into the larval habitats were formulated from the active extract and tested for stability and larvicidal activity.

RESULTS: Chloroform extract of *Cleome viscosa* Linn. and petroleum ether extract of *Artocarpus lakoocha* Roxb. were active for pesticidal activity. In bioactivity guided isolation, three compounds from *Cleome viscosa* Linn. viz., Isorhamnetin 3-O- β -D-glucopyranoside; 1- S- [N-(sulonatoxy) but- 3 -nimidoyl] -1 -thio- β - D glucopyranose or Sinigrin; 5- hydroxy 1, 4 naphthoquinone di-one- 6- β - D glucopyranose or Juglone-3-D- glucopyranose and three compounds- Cycloartenol, Lakoochin A; and Albafuran B from the leaves of *Artocarpus lakoocha* Roxb., were

been isolated, identified and are being reported for the first time even though they were previously reported from the other parts of the plant and other species of the family. A higher value Predator safety factor (PSF) of active extracts, than isolated compounds on *Poecilia reticulata* indicates the safety for concomitant use in presence of a nontarget species. Sinigrin, an ally isothiocyanate was selected as biomarker for larvicidal activity for formulation development with the chloroform extract of *Cleome viscosa* Linn. Formulations F4, F5, and F10, with the best physicochemical property and larvicidal activity, were subjected to stability studies. Floating tablet F4 and wettable powder F10 were more stable with significant larvicidal activity than liquid concentrate F5.

CONCLUSIONS: All three selected mosquito species were susceptible to the pesticidal effects of the aerial parts of *Cleome viscosa* Linn. and the leaves of *Artocarpus lakoocha* Roxb. Six compounds from *Cleome viscosa* Linn., and *Artocarpus lakoocha* Roxb leaves were isolated and identified for the first time. The active standardized extract were formulated into stable eco-friendly formulations that can be commercialized with safe application in the presence of non-target species.

Keywords: *Cleome viscosa* Linn; *Artocarpus lakoocha* Roxb; bioactivity; larvicidal; oviposition deterrence; IGR-Juvenile hormone-mimicking activity; bioactivity guided isolation; larvicidal compounds; characterization; nontarget organisms; *Poecilia reticulata*; formulations; stability testing.

INTRODUCTION

Mosquitoes transmit parasitic and viral infections to millions of people such as malaria, dengue, chikungunya, filariasis, yellow fever, and other arboviruses with substantial morbidity and mortality¹. According to WHO, sudden outbreaks in endemic areas cause millions (over 2.5 million) of deaths annually².

Lifestyle changes, urbanisation, and travel have increased mosquito breeding grounds contributing to disease outbreaks³⁻⁸.

In the life cycle of mosquitoes, the blood meal induces essential proteins and nutrients to eggs. Accordingly, mosquito species feast on mammalian blood to derive nutrients⁴. Female mosquitoes suck blood using delicate stylets in the proboscis and teeth in the mandibles. The mosquito saliva modulates host immune response and does not cause any immunological reactions. The saliva that is left behind induces the host immune response that activates mast cells and interleukins to cause bumps and induce pain⁵.



Figure No. 1: Feeding mechanism of mosquitoes on the epidermis of skin ⁵.

The primary species include- *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*. Table No. 1 depicts the transmitted disease by the vectors.

Table 1: Epidemiological Statistics of incidence of vector-borne diseases reported by WHO ⁶

Disease	Vector	Causative agent	Incidence
Dengue	<i>Aedes aegypti</i> , <i>Aedes albopictus</i>	<i>Viral strains: DEN-1 virus, DEN-2 virus, DEN-3 virus, DEN-4 virus,</i>	50–100 million infections /year
Malaria	<i>Anopheline species</i>	<i>P. falciparum</i> <i>P. vivax</i> , <i>P. malariae</i> , <i>P. ovale</i>	2018: 207 million cases
Chikungunya	<i>Aedes aegypti</i> , <i>Aedes albopictus</i> .	<i>Alpha virus</i>	2014-2018: 272 000
Lymphatic filariasis	<i>Anopheline species</i> <i>Aedes species</i> <i>Culex species</i>	<i>Wuchereria bancrofti</i> , <i>Brugia malayi</i> , <i>Brugia timori</i>	120 million infections, 40 million disfigured and incapacitated
Yellow fever	<i>Aedes species</i>	Viral hemorrhagic disease	200 000 cases causing 30,000 deaths,
Japanese encephalitis	<i>Culicine species</i>	JE virus (JEV), Flavivirus	2017–2018: 27 059 cases
Zika	<i>Aedes species</i>	Zika virus	NA
Rift Valley fever	<i>Aedes species</i>	Rift Valley virus (RVV)	NA
West Nile fever	<i>Culicine species</i>	<i>West Nile virus (WNV)</i>	NA

With such high disease incidences, strategic mosquito controls that affect the mosquito lifecycle should be implemented.

Strategic mosquito control methods⁸⁻¹⁰

Physical & physico-chemical control: Minimizing exposure, use of repellents, Use of insecticide-treated mosquito nets, physical barriers.

Biological control: *Larvivorous fish and copepods, bti- bacillus thuringiensis*, a strain of bacteria that produce, spore toxins specifically target mosquito larvae.

Chemical control:

Pyrethroid pesticides: Permethrin, Resmethrin; ***Organophosphates:*** Malathion, Clorpyrifos, Diazinon, Azinphos, Parathion; ***Carbamates:*** Aldicarb, Carbofuran, Carbaryl, Ethienocarb, Fenobucarb; ***Organochlorines:*** Di-chloro-diphenyl-ethanes, Cyclo-dienes, Chlorinated benzenes, Cyclo-hexanes and by using indoor residual spraying and Long-lasting insecticidal nets/LLINs.

In vector control, larvicidal agents are used to control the larval stage of mosquitoes. The larval control is the choicest of methods due to facts of long larval period (2-8 days) and ease of access to larval habitats. Insecticides such as DDT, and synthetic pyrethroids were favourable due to their speedy action and easy application¹¹⁻¹².

Mechanism of Insecticidal Action: The different classes of insecticides act by various mechanisms. The effects are usually found on the nervous system by a) Block of Na⁺, K⁺, Ca²⁺ channels b) Enzymatic hydrolysis of Acetylcholine and other neurotransmitters.

Figure No. 2 depicts the possible mechanism of insecticidal action in the insect neuro-humoral system which is similar to that of humans¹³⁻¹⁵. Their extensive use has led to health concerns of abnormal growth, development, impaired neurobehavioral development, cancer, and immune suppression¹⁶⁻¹⁷.

Insecticide Resistance: With the progressive and uncontrolled use of pesticides and insecticides in vector control, resistance development has developed in recent years¹⁸. These include reduced penetration- enhanced protein and lipid content in the cuticle; insensitivity of target site: nerve and ache insensitivity; reduction in mid-gut target binding site and superior detoxification.

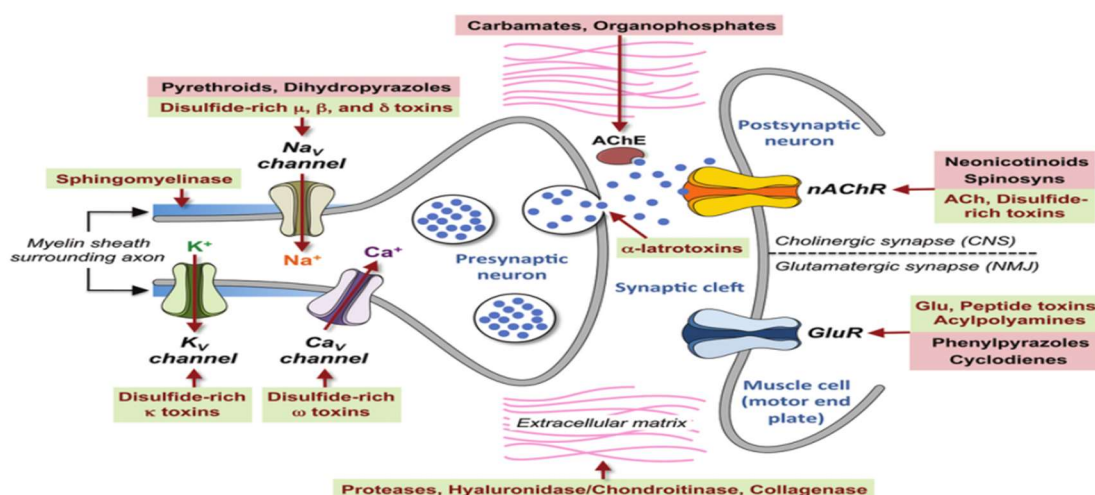


Figure No. 2: Mechanism of Insecticidal Action¹³

Overall, the disadvantages of synthetics surmount their effectiveness. Researchers are compelled to look for pest control agents that are cost-effective, eco-friendly, biodegradable & typically do not pose any threat to ecological balance^{12, 25, 26}.

In light of these challenges, researchers have turned to plant-based solutions due to their eco-friendly nature^{12,15,27}. The phyto-constituents i.e. plant secondary metabolites, have numerous biological activities. Plants have evolved with chemical defences against insects, pests, and animals. Over 2000 species, with valuable active metabolites have been identified, in pest control programs²⁷⁻³⁰. Plants and their extracts can be used as larvicides, insect growth regulators, repellents, oviposition deterrents, and attractants²⁸. Plants such as Neem²⁹, Chrysanthemum species³⁰ rotenone^{12,32} and tobacco³³ are biopesticidal due to the facts that they are biodegradable, inexpensive, and less likely to cause ecological damage and develop resistance. Hence, plant-based insecticidal screening is the gradual thrust area of research in vector control³⁴.

LITERATURE REVIEW

For this research, aerial parts of *Cleome viscosa* Linn. (CVA) & the leaves and fruits of *Artocarpus lakoocha* Roxb. (AL) were selected. This section details the salient features observed after an exhaustive literature survey on the above-mentioned plants, their biological activity, larvicidal activity, oviposition deterrence, insect growth regulatory juvenile hormone mimicking activity, safety of non-targets, and formulation development.

Table No. 1a: Literature survey of plants, activity, isolation and formulation development

Activity	Key literature findings	Reference
Vector Borne Diseases	High incidence of mosquito vector borne diseases: Mosquitoes transfer malaria, dengue, chikungunya, filariasis, yellow fever, and other arboviruses. These diseases cause morbidity, mortality, and economic loss with disruptive social life. Hence there is urgent need to control the vectors.	1-2,8-10
Use of synthetic pesticides	Biohazards of synthetic pesticides: Human health concerns & biohazard to the environment due to extensive use of synthetics, including abnormal growth, development, neurobehavioral impairment, cancer, and immune suppression.	13-16, 20-21
Resistance to pesticides	Mosquito vector resistance to synthetic pesticides: Synthetics are favourable because they are easily applicable and speedy in action but are highly toxic. With progressive and uncontrolled use, the threat of resistance development has been encountered.	19-24

Plants a vector control agent	Plants are unlimited and unexplored treasurers of rich bioactive molecules that are utilized by mankind for various purposes. Thus, plants can be used as vector control agents. Since they are easily biodegradable, inexpensive, and less likely to alter the ecological balance and develop resistance, a large number of plants are being tested for their insecticidal activities against mosquitoes.	25-34
Pharmacognostic and Folklore use	Common name, key identification characters of plant, biological activity of plant <i>Cleome viscosa</i>	69
	For tooth ache, anthelmintic, stomachic, diuretic, cardiac stimulant	41-42, 49-50
Isolation of compounds	Di-terpene cleomaldeic acid and bi-cyclic diterpene-cleomeolide ⁴³ , unsaturated embrane acids (1R,3E, 7Z,12R)-20-hydroxycembra-3,7,15-trien-19-oic acid and -17,20- dihydroxycembra-3,7,15-trien-19-oic acid	44
Isolation from seeds	Coumarin-lignans- Cleome viscosin A, B, C, D (C ₂₁ H ₂₀ O ₉), 0.1% viscosic acid, 0.04% viscosin- a flavone (C ₁₅ H ₉ O ₅ -O-CH ₃) ⁴⁵ , tannins, reducing sugars, myristic acid ⁴⁶ , oleic, stearic, linolenic, palmitic acids	47-48
Pharmacognostic	<i>Artocarpus lakoocha</i> : common names, synonyms, Aphrodisiac in unani and ayurveda. Infusion of bark for wound healing; fruits as tamarind; Timber resistance to ants, termites, and wood borers of <i>Teredo</i> species	51-53

Isolation of compounds	<i>Artocarpus lakoocha</i> : Stilbenoids, arylbenzofurans, and lectins such as Artocarpin- a 3-prenylflavone and isolectin agglutinin (ALA- artocarpus lakoocha agglutinin, tetra-hydroxy-flavone, and tetra hydroxy stilbene/oxyresveratrol	54-58
Bioassay: Mosquito larvicidal activity	<i>Foeniculum vulgare</i> : extraction, solvent effects, herb: solvent ratio, mosquito species selection: <i>Culex Pipiens</i> . LC ₅₀ of 40 mg/L in the 4 th instar. Pupal toxicity @ 200 mg/L. Phenylpropanoids- active larvicidal compound	59-60
	Compound: tetra decanoic acid; LC ₅₀ values: 14.08 ppm and 25.01ppm on larvae of: <i>Aedes aegypti</i> L. And <i>Culex quinquefasciatus</i>	61
	<i>Impatiens balsamina</i> , tested on <i>Anopheles stephensi</i> and <i>Ae aegypti</i> . Out of 4 successive solvent extracts; methanolic extract had LC ₅₀ of 98.04, 119.68, and 125.06 mg/L	62
	<i>Ocimum sanctum</i> , <i>Ocimum basilicum</i> , and <i>Ocimum gratissimum</i> , tested on laboratory-reared and field-collected <i>Aedes</i> larvae. Actives- triterpenoids and sterols of essential oils and PE extracts effective with low LC ₅₀ values	63
Oviposition deterrence	<i>Cassia obtusifolia</i> leaf extract; <i>Anopheles stephensi</i> , Terpenoids and alkaloids had effective oviposition deterrence and skin-repellent properties	64

Oviposition and ovicidal efficacy	Root of <i>Argemone mexicana</i> , <i>Aedes aegypti</i> . Non-polar extracts effective than polar extracts. Effective PE extract with 21% at 40 ppm and 100% at 1000 ppm. Forced egg retention, Ovicidal effects after 24hrs exposure of hexane and PE extract with only 4.9 and 8.9% of eggs hatching at 1000 ppm	65-66
IGR/ JH mimicking assay	Juvenile Hormone analogs (JHA) useful in mosquito control. JHA does not induce instant mortality, but effects marginal stages of larvae as they serve as food sources for fish and insects. JHA effects are mild, transient, highly acceptable for their non-toxic and reversible effect on most aquatic arthropod	67-69
	<i>Abutilon indicum</i> : Effects on Larvae, pupa and adults. Hexane extract - LC ₅₀ value of 261.31 ppm at 24 hrs. Prolonged exposure with effects on intermediates with deformities and growth index	70
Isolation of Phyto constituents	Isolation leads to reproducibility, dose fixation, and therapeutic index for <i>in-vitro</i> and <i>in-vivo</i> protocols, scientific validation of assay methods and rationalization, characterization, and establishing the chemical nature. Thus, isolated compounds can serve as marker compounds. Hence the analytical markers serve solely for analytical purposes, and active markers serve solely for specific biological activity	35-6
Isolated larvicidal compounds	<i>Zanthoxylum lemairei</i> (Rutaceae) alkaloids, 10-O-demethyl-17-O-methylisoarnottianamide, 6-acetyl-N methyl-dihydrodecarine, nitidine, and chelerythrine tested on <i>Anopheles gambiae</i> . Larvicidal activity: % mortality of 96.7%, 98.3%, and 28.3% at 250 ppm	71

Isolated larvicidal compounds	DCM extract of <i>Lanata viburnoides</i> was fractionated with hexane, ethyl acetate, and methanol. Furano-naphthaquinones regio- isomers, lantadene triterpenoid- camaric acid, lantadene triterpenoid- camaric acid with $LC_{50} = 5.48, 6.19, < 10$ ppm at 72 hrs	72
Bioassay guided fractionation	PE extract of <i>Zingiber officinale</i> : 4-gingerol, 6-dehydro gingerdione, and 6-dihydro gingerdione with LC_{50} 4.2, 9.8, 18.2 ppm on <i>Aedes</i> and LC_{50} 5.5, 7.6, 27.2 ppm on <i>Culex</i> . Characterization revealed that 2 & 3 were new compounds previously not reported	73-74
	<i>Clausena anisate</i> 1,2-benzenedicarboxylic acid, mono-(2-ethylhexyl) ester, EA extract eluted with hexane & chloroform to get 27 fractions. Fraction F2 was separated to get 40 subfractions. F2 sub-fraction eluted with hexane to 20 sub-sub fractions. Fractions 3-4 with the single compound in TLC as white amorphous powder and were subjected to spectral studies. Compound larvicidal activity with LC_{50} of 8.94, 9.23, 12.067 ppm on <i>Aedes aegypti</i> ; <i>Anopheles stephensi</i> , <i>Culex quinquefasciatus</i>	73
	<i>Tabebuia avellanedae</i> bark 2-hydroxy- 3(3-methyl-2- butenyl)1,4-naphthalene dione effective on <i>Aedes Aegypti</i> , <i>Culex Papiens</i> , and <i>Ochlerotatus togoi</i> . Other compounds ,4-naphthalene dione, 1,2-naphthalenedione, 1,4-naphthalenediol, 2-chloro-3-pyrrolidino-1,4-naphthalenedione, 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalene-dione, 2-chloro-3-morpholino-1,4-naphthalene-dione had LC_{50} values of 1.26,1.43, 3.20, 5.11, 8.30, 12.98 ppm against <i>Culex pipiens</i> larvae	74-75

Ecological safety:	When pesticides are applied in mosquito larval habitats, the other organisms are also affected. Organisms such as arthropods- <i>Toxorhynchites splendens</i> , <i>Ephemeroptera</i> - may fly, <i>Therosets</i> - diving beetles <i>Diplonychus indicus</i> , <i>Anisops bouvier</i> , midges, cephalopods- snails, amphibians- frogs, toads, salamander; fishes- <i>Gambusia affinis</i> , <i>Poecilia reticulata</i> become easy targets of larvicidal agents and pesticides applied. Hence, the toxicity of the pesticides has to be quantified before using concomitantly.	
	Non-target species-. <i>Toxorhynchites splendens</i> , <i>Gambusia affinis</i> , <i>Diplonychus indicus</i> , and <i>Poecilia reticulata</i> , habitat along with mosquito larvae. The suitability index/ Predator safety factor was calculated for the concomitant use of extracts/ isolated compounds in the presence of predatory species. Higher the SI value, greater safety. Hence integrated Vector Control and management protocols	61-62
	Pesticidal Plant extracts: <i>Atlanta monophylla</i> , <i>Rhinacanthus nasutus</i> , <i>Cuminum cyminum</i> , <i>Allium sativum</i> , <i>Zingiber officinale</i> , <i>Curcuma longa</i> and <i>Solanum tuberosum</i> . Tested non target species: <i>Toxorhynchites splendens</i> , <i>Gambusia affinis</i> , <i>Poecilia reticulata</i> , <i>Diplonychus annulatum</i> , and <i>Chironomus circumdatus</i> . Mortality and abnormality observations include sluggishness and reduced swimming up to 72 hr	76-78
Formulation & safety on non-targets	<i>Rhinacanthus nasutus</i> , tablet formulation 5% and 10% tablets; <i>Aedes Aegypti</i> with 48 hrs LC ₅₀ values of 13.6 and 14.2 mg/l for the 5% and 10% tablets. <i>Culex quinquefasciatus</i> were with LC ₅₀ values of 18.7 and 17.3, ppm. Acute toxicity LC ₅₀ values were 5-10 times greater than larvicidal dose. This indicated the biosafety of product.	78

Isolation and larvicidal formulations	The contact toxicity of isolated flavonoids, fatty acids, and 12 formulations of methanol extract of <i>Millettia pinnata</i> was evaluated, on <i>Aedes aegypti</i> and <i>Culex Pipiens</i> . Potent toxicity of flavonoids and fatty acids were at LC ₅₀ of 14.61–28.22 ppm and 16.13–37.61 ppm	79
Formulation development & Commercialization	Plant products typically end up as extracts at the laboratory scale and, for practical field applications, they need to be formulated in convenient forms. WHO recommends the use of insecticides in various forms in concentrations of 2- 80%. Commercial formulations are easily modified, prepared, and applied more feasibly in larval habitats. Plant-based larvicidal tablets, liquid concentrates, and wettable powders were prepared. The formulations can be tested in the laboratory or fields, to establish their toxicity and stability	39-40
	Larvicidal efficacy of neem oil concentrate with polyoxyethylene ether, sorbitan dioleate, and epichlorohydrin, were tested against laboratory and field- collected <i>Anopheles tsephensi</i> , <i>Culex quinquefasciatus</i> . After stability studies, larvicidal bioassay reported LC ₅₀ of 0.35 and 0.69 ppm on the two species. Field trials indicated that the mortality was also higher than <i>Culex</i> Sp. With profound growth inhibition effects and pupal mortality Aksebio2, botanical water-suspensible materials prepared from aromatic essential oils (thyme, oregano, anise), edible sesame and maize oil, and compounds carvacrol, thymol and, anethole. Mortality greater than 90% was observed at 12.5, 25, and 50 ppm at 24 hrs I and II instars <i>Culex pipiens</i> .	80-81
	Hexane, chloroform, ethyl acetate, acetone, methanol, and aqueous leaf extracts of <i>Nelumbo nucifera</i> Gaertn. (Nymphaeaceae) were synthesized as silver nanoparticles and tested on larvae of <i>Anopheles subpictus</i> and <i>Culex</i>	82

	<i>quinquefasciatus</i> Say. LC ₅₀ =8.89, 11.82, and 0.69 ppm on <i>Anopheles subpictus</i> and LC ₅₀ of 9.51, 13.65, and 1.10 ppm on <i>Culex Quinquefasciatus</i> suggest the effectiveness of <i>N. Nucifera</i> in the preparation of ideal eco-friendly formulations for mosquito control	
	Natular® contains natural insecticide spinosyn A and spinosyn D from the bacterium <i>Saccharopolyspora spinosa</i> . Natular® is a matrix tablet with water-soluble polyethylene glycol (PEG) and water-insoluble calcium sulfate hemihydrates that enables slow dissolving, exposure, and release of the spinosyns, which is effective against mosquito larvae	83-84
	Wettable powders contain 5 to 95 percent active ingredients that do not dissolve in water but are suspended in water. Wettable powders are one widely used formulations with the advantages of ease to store, transport, and handle, non-toxic with low skin and eye absorption than liquid formulations	85
Stability testing of formulation	In the stability testing of tablets, parameters such as appearance, color, odor, assay, dissolution, moisture, and friability are to be evaluated. For emulsions, evaluations according to stability testing of drug substances and drug products by the US-FDA include Appearance (including phase separation), Color, Odor, Assay, Degradation products, pH, Mean size, Viscosity, Microbial limits, Preservative content, Distribution of dispersed phase globules	86

2.1: Critical analysis of literature gap to research objectives

Mosquitoes are major vectors of deadly diseases like malaria, dengue, Zika, chikungunya, and yellow fever, causing millions of infections and deaths globally. Insecticide resistance, environmental concerns, and health risks from synthetic chemicals highlight the need for natural mosquito control agents. Botanical extracts, microbial larvicides, and biocontrol methods like predatory fish offer eco-friendly, sustainable alternatives. These natural solutions help reduce mosquito populations while minimizing ecological disruption and human health hazards.

The discovery of novel pesticides like azadirachtin, derived from neem, is crucial for sustainable pest management. plant based pesticides are biodegradable, eco-friendly, and disrupts insect growth and reproduction without harming beneficial organisms. Biopesticides offer safer, long-term solutions for mosquito and agricultural pest control.

Literature survey mentioned use of selected plants for traditional use and certain biological activities. the potential of larvicidal activity and larval toxicity studies were not explored for *Cleome viscosa* while only the preliminary screening for larvicidal activity was reported on AL. Oviposition deterrence, JH activity were considered for possible mechanism of pesticidal action. the

Extraction techniques: The solvents selection in literature is either one or two solvents only. But to understand the true nature of larvicidal compounds, selection of solvents and extraction methods are to be done critically. Successive method of extraction was adopted to separate the phytoconstituents based on their polarity.

Isolation of compounds: Specific compounds for the larvicidal compounds were not documented in literature from the selected plants. Bioactivity guided isolation for larvicidal activity were also not reported. Bio markers of isolation would serve as first step towards standardisation of extracts and forms the basis for formulation development.

Ecological safety profiling: Larvicides are concomitantly applied with other species of organisms in water bodies. To confirm on target specificity and to establish safety profile to other organisms a non-target organism- *Poecilia reticulata*, a larvivores fish was selected for the study.

Formulation development: Plant extracts usually end up only as extracts in laboratory and do not reach to field trials or commercial scale production due to various factors like nature, yield, scalability.

As a first stepping stone, standardisation of extracts and its formulation development were part of the study. In the formulation development section, the aim was to convert biologically active herbal extract into feasible formulation that is convenient for application.

Also, the biomarkers of isolation would serve as for internal standards for formulation development. Stability studies of formulation, ensure the stable nature of formulation after development and would be the baseline for commercializing extracts and its formulations.

JUSTIFICATION OF THE STUDY

The present study was aimed at bioactivity-guided isolation of compounds for mosquito larvicidal activity from plants *Cleome viscosa* Linn., and the *Artocarpus lakoocha* Roxb., and to develop herbal formulations.

Study criteria	Justification
Mosquitoes as vectors of diseases	Mosquitoes as vectors of diseases
The larval stage of mosquitoes	Easy to access for application of larvicidal agents Vertical transmission of micro-organisms can be controlled
Existing synthetic pesticides	Bio-hazardous, Mosquito resistance development, Effects on non-targets, Ecological imbalance
Plant selection	Traditionally used, Phytoconstituents for insecticidal activity not identified, Plant extract has not been standardized
Bioactivity guided isolation	Biomarkers for insecticidal properties are not identified.
Formulation development	Academically, extracts are proven for efficacy and are not developed further. Application of Extracts is not feasible in mosquito breeding sites Hence, they need to be formulated with the possibility of commercialization

With these needs for the study, primary & secondary objectives were postulated.

The primary objectives were:

- To isolate the active larvicidal components from
Ø Artocarpus lakoocha Roxb.
Ø Cleome viscosa Linn.
- To identify the mechanism of larvicidal action of extract & isolated compounds
- To develop an eco-friendly plant-derived pesticidal formulation from *Artocarpus lakoocha* Roxb. / *Cleome viscosa* Linn.

The secondary objectives were:

1. Collection and authentication of plant materials.
2. Pharmacognostical standardization of plant materials and extracts.
3. Maintenance and rearing of mosquito larvae of primary vector species- *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*.
4. Determination of larvicidal, Oviposition deterrence, and Juvenile hormone-mimicking- IGR activity of successive solvent extracts on selected larval species.
5. Statistical evaluation of pesticidal activities- LC₅₀ and LC₉₀ values of extracts.
6. Identification of the most active extract with significant larvicidal activity in comparison to standard Azadirachtin (Neem).

MATERIALS AND METHODS

2.1: Collection and Authentication of Plant Material

The *Cleome viscosa*, *Artocarpus lakoocha* Leaf, and *Artocarpus lakoocha* Fruit were collected in the fruiting and flowering seasons from the Western Ghats. All the plant raw materials were identified and authenticated with a plant taxonomist at Natural Remedies Pvt. Ltd, Bengaluru. After botanical identification, voucher specimens (223/2014, 224/2014, 221/2014 for leaves and fruits of *Artocarpus lakoocha* Roxb. and aerial parts of *Cleome viscosa* Linn.) were labelled and deposited at the herbarium of the Department of Pharmacognosy, KLE College of Pharmacy, Bengaluru.

2.2: Pharmacognostical Evaluation of Plant Material

Pharmacognostical evaluations of the powdered drug, such as organoleptic, physicochemical, microscopical evaluation, fluorescence analysis, and phytochemical analysis were as per standard procedures⁸⁷⁻⁸⁸. Microscopical evaluations were as per standard procedure⁴¹. The picto-micrographs were recorded on a microscopical camera. Physicochemical evaluation and fluorescence analysis were to understand the nature of phytoconstituents and their behaviour under UV light. Phytochemical screening of successive solvent extracts was carried out with chemical reagents to identify the phytoconstituents in the extracts as per standard procedures. Successive solvent Extraction was done by placing # 60 powder, packed as a thimble, placed in Soxhlet assembly, and successively extracted with four solvents in the increasing order of polarity viz. petroleum ether, chloroform, ethanol, and water. Solvent selection was to separate the phytoconstituents in the increasing order of polarity. After the effective extraction for 48 hrs, the solvent was distilled off by concentrating in Buchi Rotavapor and stored at 4± 1.0 °C temperature until the bioassay. The physical nature of extracts viz., color, consistency, odor, and percentage yield of the extracts was recorded.

2.3: Collection and Laboratory Maintenance of Mosquito Species

Anopheles stephensi (RS-Bengaluru strain), *Aedes aegypti* (Bengaluru strain) and *Culex quinquefasciatus* (Bengaluru strain) were representative of each mosquito species since the spread of the diseases are based on the population dynamics of these critical species. Eggs of *Anopheles stephensi*, *Aedes aegypti* and egg rafts of *Culex quinquefasciatus* were collected around Yelahanka, Bengaluru, and transferred into separate enamel pans (30 x 25 x 4 cm) of 2 L capacity. The larvae were fed on a powdered mixture of dog biscuits and dried yeast powder at a ratio of 3:1 until growth into 3rd instar larvae and maintained under a photo regime of 14:10 (L: D) and a temperature of $27 \pm 2^\circ\text{C}^{89}$.

2.4: Larvicidal Bioassay

Larvicidal bioassay of the successive solvent extracts of *Cleome viscosa*, *Artocarpus lakoocha* Leaf, and *Artocarpus lakoocha* Fruits were carried out according to **WHO Pesticide Evaluation Scheme WHO/CDS/WHOPES/GCDPP/2005**. To prepare a 1% v/v stock solution, aliquots of DMSO (0.1- 0.5 ml) were added to dissolve extracts, and the volume was marked up with distilled water. Aliquots of the stock solution (1% w/v solution) were transferred into a measuring cylinder; the volume was made up to 250/ 100 ml using tap water. The solutions were transferred into glass beakers/ test plastic bowls of 500 ml capacity. Water and 10-500 ppm of 0.01% v/v azadirachtin served as a negative and positive control.

Twenty-five healthy late 3rd/ early 4th instar larvae of each species were picked and dropped gently into test bowls using strainers. Time, temperature, RH, and L:D of each experiment were noted. Affected larvae were identified as dead when they failed to move after probing with a needle in the siphon or cervical region. Larvae, that were incapable of rising to the surface and did not show the characteristic diving reaction

when the water was disturbed are identified as moribund/affected larvae. The mortality was recorded hourly for the first 6 hrs, then for every 6 hrs up to 72 hrs of the exposure period. Results from replicates were recorded and pooled for probit analysis as recommended in the protocol¹²⁸. LC₉₀ values are determined using toxicity studies. For toxicity studies, 5-10 concentrations of test solutions below LC₅₀ values of preliminary screening were prepared and tested with the same procedure mentioned above.

2.5: Oviposition Deterrence Activity

The aliquots of the 1% v/v stock solution of extracts of CVA, ALL and ALF (50-500 ppm) were used for larvicidal activity were used similarly for oviposition deterrence assay in concentration range of 50-500 ppm. Fifteen healthy gravid female mosquitoes that are less than 10 days old and fed with blood 4 days prior) were transferred to small mosquito cages with placement of positive, negative control and test solutions placed in opposite corners of each cage. The bowl positions are to be altered between three replicates to nullify the effect of position. After 24 hrs, appearance, color, and the number of eggs in each test and control were noted. The percentage effective repellence (% ER) were calculated using the formula: $ER \% = \frac{NC - NT}{NC} \times 100$, Where, ER- Effective repellence; NC- Number of eggs in control, NT- Number of eggs in treatment. The results from all replicates were recorded and pooled for probit analysis.

2.6: Juvenile hormone-mimicking – Insect growth regulatory (IGR) activity

The juvenile hormone-mimicking activity of the PEE, CHE, ETE, and WE of CVA, ALL, and ALF were carried out according to WHO Pesticide Evaluation Scheme WHO/CDS/WHOPES/GCDPP/2005126 like larvicidal activity below LC₅₀ values of preliminary screening. The growth-inhibiting potential of extracts such as pupal morphological abnormalities and partially emerged adults and completely emerged adults and others were recorded until all the pupae in control bowls emerged. The

percentage of emergence inhibition (% EI) in the treated and control were calculated for 6, 12, 18, 24, 48 hrs and until complete emergence in the negative control group. The results from all replicates were recorded and pooled for probit analysis.

2.7: Bioactivity-Guided Isolation of Larvicidal Compounds from *Cleome viscose*, & *Artocarpus lakoocha* Leaf

The bioactivity-guided isolation of larvicidal compounds was carried out for **CHE of *Cleome viscose*, and PEE of *Artocarpus lakoocha* Leaf**. 12.0 – 15.0g of extracts was fractionated by flash chromatography Combiflash Rf 200 psi over Redisep Rf 150 g silica gel preppacked flash column. The column was eluted with a combined solvent of petroleum ether and ethyl acetate in increasing polarity (from 100-30%). 50 ml fractions were collected and observed by TLC (pre-coated, 0.02 mm thick, E. Merck, Germany 60 F₂₅₄). Fractions with similar TLC pattern were pooled, concentrated based on TLC pattern of crude extract and concentrated in rotavapor at 30°C and 25rpm. fractions were subjected to larvicidal activity at 100 ppm. Active fractions were further purified to get pure isolated compounds. The four isolated compounds were subjected to larvicidal bioassay at 10-50 ppm in triplicates. Mortality was recorded hourly for the first 6 hrs, then for every 6 hrs up to 72 hrs. Probit analysis on the 24 hrs mortality was used to calculate LC₅₀ values. **The isolated compounds were subjected to characterization by chromatographic and spectroscopic methods¹²⁹⁻¹³².**

2.8: Characterization and Structural Elucidation of Isolated Compounds:

UV absorption spectra were determined on a double-beam spectrophotometer, Shimadzu 1700, IR spectra by FT-IR 460 with basic unit & software, Jasco, KBr disc method, 1D and 2D NMR spectra were recorded at 400 MHz using AV 400 Bruker 400 MHz high-resolution multinuclear FT-NMR spectrophotometer in DMSO and ECX400 Jeol high-resolution multinuclear FT-NMR spectrophotometer using TMS. Mass

spectroscopy was on Agilent LC/MS, TOF mass spectrometer. HPTLC fingerprint on TLC plates using CAMAG Linomat IV sample applicator (Muttentz, Switzerland). Flash column chromatography was on Combiflash Rf 200 psi (Teledyne, USA) with UV-vis (200–800 nm) and a secondary wavelength monitoring system. HPLC method of Analysis was developed on Shimadzu HPLC system, LC 2010 A with UV and PDA detector, LC solution software. RP C18, Shodex 5 μ m, 250 X 4.6 mm. Gradient elution was carried out with potassium di-hydrogen orthophosphate in water as solvent A and Acetonitrile as solvent B. 20.0 μ l of the prepared sample solution was injected at intervals of 55 minutes and scanned at wavelengths of 210, 254, and 280 nm. The retention time and percentage purity were determined from the chromatograms.

2.9: Effect on Non-target Organisms

Twenty fishes (length 1.5- 3.5 cm) were exposed to 10-500 ppm of isolated compounds/ extracts ($> LC_{90}$ values of target mosquito larvae). The swimming behaviour and survival were recorded for three days at 24 hrs intervals. LC_{50} values were calculated by probit analysis¹¹⁵⁻¹¹⁸. The Suitability index (SI) or Predator safety factor (PSF) of extracts and isolated compounds was calculated using the formula: $SI = LC_{50}$ of non-target species / LC_{50} of target species¹⁰².

2.10: Development of Insecticidal Formulations

The CHE of *Cleome viscosa* with good larvicidal activity and suitability index were formulated as a) Floating tablets b) Liquid concentrate c) Wettable powders.

2.10.1: Floating Tablets

HPMC, chitosan, and sodium alginate, effervescent components, were used for preparation of floating matrix tablets of CHE of CVA (400 mg tablets) by direct compression using xanthan and karaya gum as binder¹³⁵⁻¹³⁶. All the ingredients were blended homogeneously for 10-20 minutes, followed by addition of magnesium stearate.

Air-dried wet granules were passed through sieves # 8, # 16 & # 44 and analyzed by IR. Compatible formulation blends were punched on Rimek RSB-4 Minipress using a 12 mm flat punch.

Physico-chemical parameters of floating tablets: Weight variation test (Limits: $\pm 5\%$), friability test, hardness test, tablet thickness and diameter, content uniformity, swelling Index, *in-vitro buoyancy*/ floating studies were tested and recorded¹³⁵⁻¹³⁸.

Table No. 2: Formulation chart of floating tablets F1-4

Ingredients (mg)	F1	F2	F3	F4
CHE of <i>Cleome viscosa</i> ,	200	200	200	200
HPMC k100	50	55	50	50
Sodium alginate	50	35	50	30
Xanthan gum	35	25	30	60
Karaya gum	10	20	10	5
Tamarind seed kernel	10	20	10	5
Citric acid	15	15	15	15
Sodium bicarbonate	20	20	20	20
Pvp k-30	5	5	10	10
Mg-stearate	5	5	5	5
Total weight (mg)	400	400	400	400

2.10.2: Emulsifiable Liquid Concentrate

Emulsions- liquid concentrates were prepared as with application as sprays- indoors and outdoors⁴⁵⁻⁴⁸. The formulation chart of the Liquid emulsifiable concentrates is in Table No. 3. The CHE of *Cleome viscosa* was dispersed in oil. Primary emulsion (4: 2:1 -O: W: G) was prepared by wet gum method. Stable formulations F5–F7 were

analysed for physicochemical properties¹³⁹⁻¹⁴³. The viscosity was measured using a Brookfield viscometer at room temperature. The specific gravity and pH were determined using of 1% w/v solution in water.

Table No. 3: Formulation chart of Liquid concentrates formulations F5-F7

Ingredients (%)	F5	F6	F7
CHE of <i>Cleome viscosa</i>	5	5	5
Tween 80	23	18	25
Tamarind Seed Gum	6	2	4
Glycerine	5	2	2
Sorbitol	15	10	10
Acacia Gum	5	2	3
Oil (Sunflower Oil)	20	30	25
Gallic Acid	0.5	0.5	0.5
Sodium Benzoate	0.5	0.5	0.5
Q.S Purified Water	20	30	25
Total Volume (ml)	100	100	100

2.10.3: Wettable Powders

Water-wettable powders F8-F12 (Table No. 4) were prepared by heat melt-dispersion. CHE of CVA and different proportions of molten polyethylene glycol and tween 80, tamarind seed powder were melted with light stirring using a glass rod. The mixture was allowed to cool to room temperature naturally at 25°C and the solidified mixture was grounded in a ball mill/blender with talc, and magnesium stearate, and sieved using # 150 and stored in sealable air-tight containers. The formulations F8-12 were tested

with physicochemical characteristics like colour, odour, moisture content, and flow properties, pH of 1% solution were observed¹⁴⁷⁻¹⁵⁰.

Table No 4: Formulation of Wettable Powders F8-12

Ingredients (%)	F8	F9	F10	F11	F12
CHE of <i>Cleome viscosa</i>	20	20	20	20	20
PEG 4000	20	20	30	30	30
Tween 80	20	20	10	10	10
Tamarind seed powder	10	10	10	20	10
Talc	20	20	20	10	20
Mg stearate	10	10	10	10	10
Total Weight (G)	100	100	100	100	100

2.11: Larvicidal Activity of Formulations F1-F12

The Larvicidal activity of formulations F1-F12 were as per the standard procedure mentioned earlier. Results from all replicates were recorded and pooled for probit analysis¹²⁸.

2.12: Stability Testing of Formulations F4, F5 and F10

Out of the twelve formulations of CHE of *Cleome viscosa*, the most effective formulations, F4 of tablets, F5 of emulsifiable concentrate and F10 of powder were subjected to stability studies as per Table No. 5¹⁵⁰⁻¹⁵².

Table No. 5: Stability testing parameters selected for active formulations F4, F5, and F10

Stability testing	Temperature (°C)	RH (%)	Months
Accelerated	40 ± 2	75 ± 5	6
Intermediate	28 ± 2	75 ± 5	6

DATA ANALYSIS METHODS

With the scientific and literature background, the following null hypothesis and alternative hypothesis were considered.

H₀ (Null Hypothesis): Aerial parts of *Cleome viscosa* Linn., and leaves and fruits of *Artocarpus lakoocha* Roxb., are inactive as pesticidal agents against primary vector species- *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*.

H₁ (Alternative Hypothesis): Aerial parts of *Cleome viscosa* Linn., and leaves and fruits of *Artocarpus lakoocha* Roxb., are active as pesticidal agents against primary vector species- *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes Aegypti*.

Thus, the study aimed to prove the null hypothesis by incorporating statistical evaluation methods such as probit analysis with chi-square, standard deviation and one-way Anova as recommended in the WHO/ PES protocol¹²⁸.

Probit analysis is a specialized regression (method of fitting a line) model of binomial response variables i.e., mortality vs. concentrations using SPSS® academic software trail version No. 20. Standard deviation and one-way Anova were calculated using Graph pad prism® software 5.

Probit analysis is used to determine the relative toxicity of the extracts/ fractions/ formulations against mosquito larvae by testing the response of an organism under various concentrations encountering response and then comparing them.

The mortality and various concentrations give a sigmoid curve. Probit analysis transforms from linear to sigmoidal and then runs a regression on the relationship.

LC₅₀ and LC₉₀ values are determined with statistically significant 95% fiducial limits of upper and lower limits. Chi-square values were considered with a threshold of statistical significance at $p < 0.05$.

RESULTS

4.1: Plant Materials

4.1.1: Collection and Authentication of Plant Materials

The leaves and fruits of *Artocarpus lakoocha* Roxb. (ALL & ALF), aerial parts of *Cleome viscosa* Linn. (CVA) were authenticated (Annexure I) and key identification features of identification as in Figure No. 3.

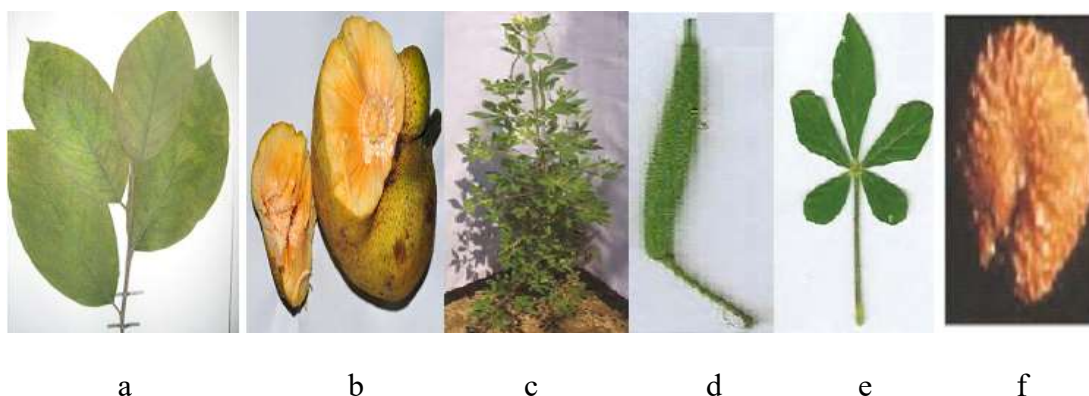


Figure No. 3: Key identification features of plant materials a) AL leaves, b) AL-fruits, and c) *Cleome viscosa*- whole plant d) fruit, e) leaf, f) seeds⁷⁹⁻⁸³

4.1.2: Standardization of Plant Materials

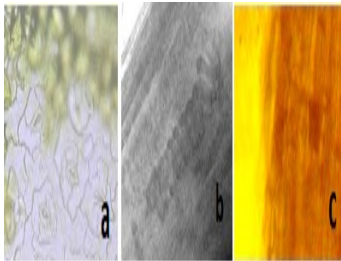
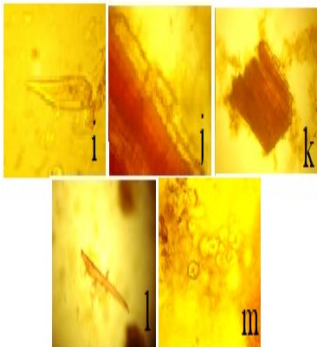
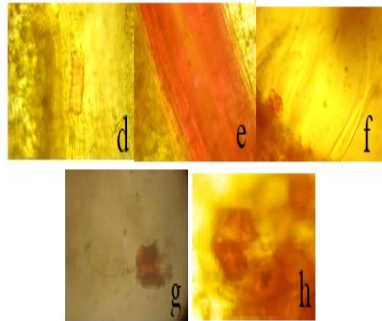
4.1.2.1: Pharmacognostical evaluation of plant materials

Organoleptic evaluation of plant materials: Physicochemical properties such as color, odor, texture, appearance, etc., were observed Table No. 6 and Figure No. 4.

4.1.2.2: Physicochemical evaluation of plant materials

The ash values, and extractive values are as in Table no. 7 and Figure no. 5. Phytochemical analysis is as presented in Table no. 8. Fluorescence analysis of the powdered drug is presented in Table No. 9 and Figure No. 6.

Table No. 6: Organoleptic characteristics of plant materials

	Crude drug	Powder #40
<i>Cleome viscosa</i>	Nature: The entire plant is pubescent Color: Green leaves, stem, and fruit pods with brown seeds; Odor: Strong penetrating; Taste: Slight bitter, Texture: Rough & fibrous; Length: 1 – 1.5 m; Width: 0.8-1.2 cm	Nature: Coarse powder # 40; Color: Dark green Odor: Characteristic; Taste: Slight bitter; Texture: Fibrous
<i>Artocarpus lakoocha</i> Leaf	Nature: Oblong, ovate, broad base, acute apex Color: Dark green shiny outer surface, pubescent inner surface. Odor: Characteristic; Taste: Slightly bitter; Texture: Rough & fibrous; Length: 5-12cm; Width: 6-9cm	Nature: Coarse powder # 40 Color: Green Odor: Characteristic Taste: Slightly bitter; Texture: Rough and fibrous
<i>Artocarpus lakoocha</i> Fruit	Nature: Characteristic sub-globule, irregularly shaped fruits; Color: Outer light green, inner bright yellow with white seeds; Odor: Characteristic; Taste: Sweet-sour, astringent; Texture: Pubescent; Length: 6-10cm;	Nature: Coarse powder # 40; Color: Dark brown; odor: Characteristic Taste: Sweet-sour, astringent; Texture: Rough
Powder CVA: anamocytic stomata (a), spongy parenchyma, spiral and reticulated vessels (b), prisms of calcium oxalate (c)  Powder: ALL: single-celled thick wall trichomes (i), phloem with lignified fibres (j, k), transparent fibres (l), starch granules with central hilum (m)  Powder ALF: vessels with annular thickenings(d), lignified fibres (e), covering trichomes (f), glandular trichomes with brown heads (g), pitted vessels, and Stone cells (h) 		

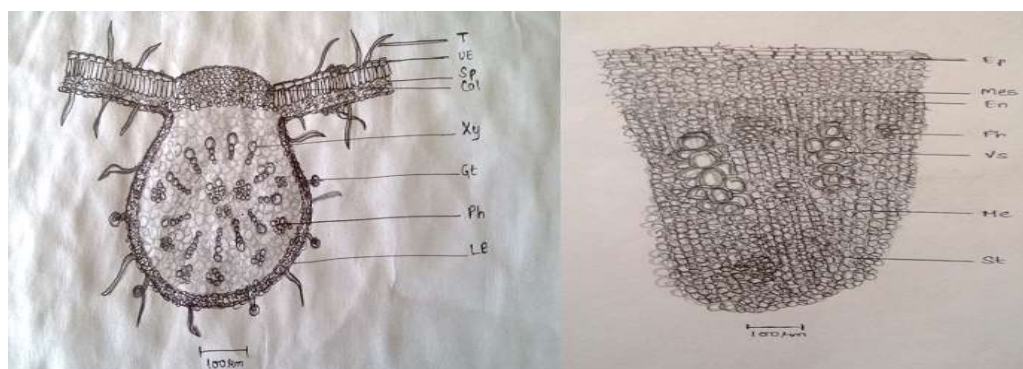


Figure No. 4: a) Transverse section of *Artocarpus lakoocha* Leaf; T- trichomes, UE- upper epidermis, SP- spongy parenchyma, Col- collenchyma cells, Xy- xylem, Gt- glandular trichomes, Ph- phloem, Le- lower epidermis b) Transverse section of fruits of *Artocarpus lakoocha* fruits; Ep- Epidermis, Mes- Mesocarp, En- endocarp, Ph Phloem fibres, Vs- Vascular tissue, ,Mes- Mesophyll, Mes, st: sclerenchymatic transmitting tissue

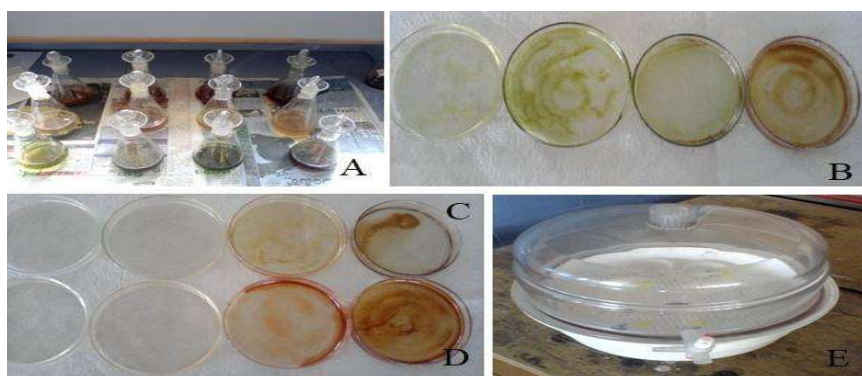


Figure No. 5: A- extracts of *Artocarpus lakoocha* Leaf, fruits, *Cleome viscose*; B- extracts of *Cleome viscose* and C, D-extracts of *Artocarpus lakoocha* Leaf and ALF; E- Total Ash o, ALF, *Cleome viscose*



Figure No. 6: Fluorescence Analysis of *Artocarpus lakoocha* Leaf, *Artocarpus lakoocha* Leaf Fruit, *Cleome viscose* extracts with solvents- hexane, pet- ether, ethyl-acetate, butanol, ethanol methanol water and Blank water, #60 mesh powder on watch glass.

Table No. 7: Physicochemical evaluation of plant materials

Plant	CV	AL	
Parameters (%w/w) $\pm$ SD	Aerial parts*	Leaf*	Fruits*
Pet ether EV	3.89 $\pm$ 0.16	6.42 $\pm$ 0.13	5.71 $\pm$ 0.26
Chloroform EV	2.19 $\pm$ 0.13	5.03 $\pm$ 0.01	9.58 $\pm$ 0.15
Ethanol EV	4.21 $\pm$ 0.16	5.99 $\pm$ 0.04	6.40 $\pm$ 0.15
Water EV	8.23 $\pm$ 0.14	10.86 $\pm$ 0.05	7.52 $\pm$ 0.16
Total ash	4.33 $\pm$ 0.15	4.07 $\pm$ 0.16	3.55 $\pm$ 0.16
Water soluble ash	0.76 $\pm$ 0.16	6.68 $\pm$ 0.13	10.24 $\pm$ 0.15
Acid insoluble ash	1.03 $\pm$ 0.12	6.35 $\pm$ 0.18	0.97 $\pm$ 0.04
Foreign organic matter	1.62 $\pm$ 0.19	0.20 $\pm$ 0.01	0.08 $\pm$ 0.01
Moisture content (LOD)	2.56 $\pm$ 0.13	3.54 $\pm$ 0.13	4.04 $\pm$ 0.14
EV- soluble extractive values, *All values are expressed as mean with SD of <5% ($p \leq 0.05$) with reference to air-dried material			

In CVA, alkaloids in chloroform extract; steroids, triterpenoids, fixed oils in PEE; tannins, flavonoids, and phenolics in ethanolic extract, and proteins and carbohydrates in aqueous extract were observed. Similarly, alkaloidal glycosides, steroids and triterpenoids, tannins, flavonoids, and phenolics in ethanolic extract of *Artocarpus lakoocha* Leaf. Proteins and carbohydrates were found in WE of ALF. The results of the phytochemical analysis are as in Table No. 8.

4.1.3: Extraction of Plant Materials

The powdered plant material (# 60) was weighed (25 g), packed as a thimble, placed in Soxhlet assembly, and successively extracted with four nonpolar and polar solvents in the increasing order of polarity viz. pet-ether 60-80 °C, chloroform, ethanol, and water. After 48 hours of effective extraction, the solvent was distilled in Buchi Rotavapor and stored at 4 ± 1.0 °C temperature until the Larvicidal bioassay. The appearance, color, consistency, odor, and percentage yields of the extracts were recorded and are presented in Table No. 10.

4.2: Maintenance of mosquito species

Three mosquito species viz., *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* were studied for various activities. Figure No. 7 represents the maintenance of larval colony at the NIMR, ICMR, Research centre, Bengaluru

Table No. 8: Phytochemical analysis of successive solvent extracts

Phytochemical test	CV				AL							
	Aerial parts				Leaves				Fruits			
	PE E	CH E	E T	W E	PE E	CH E	E T	W E	PE E	CH E	E T	W E
Mayer's test	-	+	-	-	+	-	-	-	-	-	+	-
Hager's test	-	+	-	-	+	-	-	-	-	-	+	-
Wagner's test	-	+	-	-	+	-	-	-	-	-	+	-
Dragendroff's test	-	+	-	-	+	-	-	-	-	-	+	-
Borntrager's test	-	-	-	-	-	-	-	-	-	-	-	-
Salkowski test	+	+	-	-	+	+	+	-	+	+	+	-
Liebermann Burchard	+	+	-	-	+	+	+	-	-	-	+	-
Foam test	+	-	-	-	-	-	-	-	-	-	-	-
Ferric-chloride test	-	-	+	+	-	-	-	+	-	-	+	-
Gelatin test	-	-	+	+	-	-	-	+	-	-	+	-
Ferric-chloride test	+	-	+	+	-	-	-	+	-	-	+	-
Shinoda test	+	-	+	+	-	-	-	+	-	-	+	-
Alkaline reagent test	+	-	+	+	-	-	-	+	+	+	-	-
Lignin	-	-	-	+	-	-	-	-	-	-	+	-
Molisch test	+	-	+	+	-	+	+	-	-	-	+	+
Biuret test	-	-	+	+	-	-	-	-	-	+	+	+
Ruthenium red	-	-	-	-	-	-	-	-	-	-	+	+
Spot test	+	-	-	-	-	-	-	-	-	-	-	-
+- indicates presence/positive test; - indicates absence/ negative test												

Table No 9: Fluorescence analysis of plant materials

Plant	<i>Cleome viscosa</i>			<i>Artocarpus lakoocha</i> Leaf					
Plant part	Aerial parts			Leaves leaf			Fruits		
	Observation under			Observation under			Observation under		
	WL	254nm	366nm	WL	254nm	366nm	WL	254nm	366nm
Powder as such	Green	G-brown	Brown	G-brown	Green	Black	Brown	Brown	Black
Control (water)	Colorless	Light grey	B-grey	Colorless	Blue F	Blue F	Colorless	Blue F	Blue F
Powder+ water	Yellow	Olive green	Y-grey	Buff color	Pale green	Black	Pale brown	Pale yellow	Dark grey
Conc. H₂SO₄	Light orange	Light yellow	Dark grey	Red	Yellow	Black	Brown	Olive green	Black
1N Hcl	Light brown	Light grey	Blue	Pale buff	Light grey	Grey	Yellow	Yellow	Dark grey
Ethanol	Brown	F green	Yellow	Pale green	Pale yellow	Grey	Buff	Pale grey	Pale grey
Methanol	Yellow	F green	Yellow	Olive green	Y-green	Grey	Pale yellow	Pale yellow	Dark grey
Me- NaOH	Pale green	Y-green	Dark grey	Bright yellow	Pale yellow	Grey	Yellow	Bright yellow	Dark grey
Et- NaOH	Yellow	Bright yellow	Dark grey	Pale yellow	Yellow	Grey	Pale yellow	Pale grey	Dark grey
5% KOH	Yellow	Bright yellow	Dark grey	Dark brown	brown	Black	Brown	Olive green	Black
Visualisation in WL- white light, F- Fluorescence, H ₂ SO ₄ - sulphuric acid, Hcl- Hydrochloric acid									

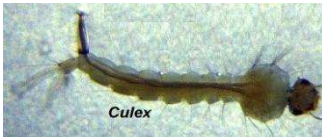
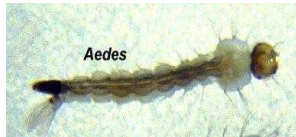
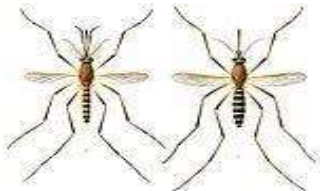
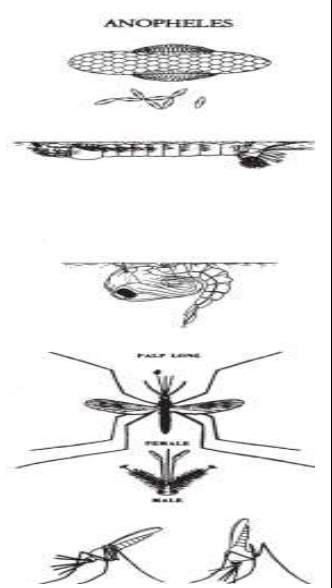
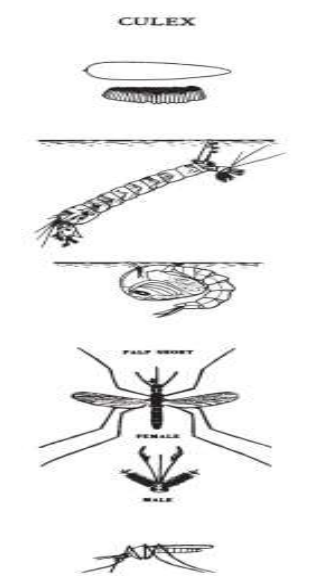
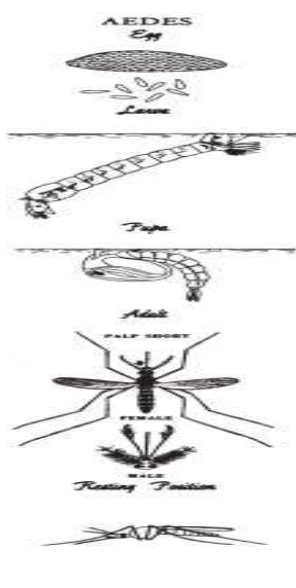
Table No. 10: Properties and Percentage Yield of Extracts

Extract E	Nature	Color	Nature	% Yield (% w/w)	Solubility	pH 1%
<i>Cleome viscosa</i>						
PEE	Oily	Green	Sticky	4.22 ± 0.01	PE, Et, DMSO	7.3 ± 0.1
CHE	Viscous	Green	Sticky	3.86 ± 0.02	Et, DMSO	7.4 ± 0.1
ETE	Viscous	Green	Sticky	3.22 ± 0.08	Me, Et, DMSO	7.4 ± 0.3
WE	Dry	Brown	Dry	4.86 ± 0.02	DMSO	7.2 ± 0.1
<i>Artocarpus lakoocha</i> Leaf						
PEE	Oily	Green	Sticky	6.35 ± 0.05	Et, DMSO	7.1 ± 0.2
CHE	Viscous	Green	Sticky	3.36 ± 0.04	Et, DMSO	7.4 ± 0.3
ETE	Viscous	Brown	Sticky	4.86 ± 0.00	Me, Et, DMSO	7.3 ± 0.5
WE	Dry	Brown	Dry	2.56 ± 0.02	Et, DMSO	7.3 ± 0.6
<i>Artocarpus lakoocha</i> Fruit						
PEE	Oily	Yellow	Sticky	5.89 ± 0.04	Et, DMSO	7.0 ± 0.2
CHE	Viscous	Brown	Sticky	3.66 ± 0.08	Me, Et, DMSO	7.2 ± 0.4
ETE	Viscous	Brown	Sticky	4.12 ± 0.07	Me, Et, DMSO	7.3 ± 0.4
WE	Dry	Brown	Dry	4.48 ± 0.08	Et, DMSO	7.1 ± 0.3
PE- pet ether 40-60°C, CH-chloroform, ET- ethanol, WE- aqueous, % w/w -% weight/weight with reference to air-dried material, All values are expressed as mean with SD of <5% (p≤0.05)						



Figure No. 7: A- Mosquito cages, B, - maintenance of larvae, C-eggs of *Aedes aegypti*, D- egg rafts of *Culex quinquefasciatus*, E- larval food, F- 2nd instar larvae, G- 3rd instar larvae, H, I- pupae

Table No. 11: Lineal hierarchy and key identification feature of Mosquito^{6, 77}

Species	<i>Anopheles stephensi</i>	<i>Culex quinquefasciatus</i>	<i>Aedes aegypti</i>
Kingdom:	Animalia		
Phylum:	Arthropoda		
Class	Insecta		
Order	Diptera		
Family	Culcidae		
Genus:	<i>Anopheles</i>	<i>Culex</i>	<i>Aedes</i>
Species:	<i>Stephensi</i>	<i>quinquefasciatus</i>	<i>Aegypti</i>
Binomial name:	Anopheles stephensi Liston, 1901	Culex quinquefasciatus Say	<i>Aedes aegypti</i> Linn.
Larvae			
Adult			
Stages			

4.3: Larvicidal Bioassay

Preliminary larvicidal bioassay:

***Cleome viscosa*:** In the preliminary screening, all the solvent extracts were tested for larvicidal bioassay (Figure No. 8). The larvicidal effect of chloroform was lower than standard azadirachtin with similar morphological toxicities as in Figure No. 9. Among the tested extracts, the CHE of CVA showed the highest larvicidal activity at the lowest concentrations of 141.992, 35.684, and 386.325 ppm on the three vector species viz., *Anopheles stephensi*, *Culex quinquefasciatus*, *Aedes aegypti* (Table No. 12 a). In the toxicity study, the CHE of CVA showed the maximum activity with LC₅₀ of 427.68, 455.92, and 634.44 ppm on *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* respectively as depicted in Table No. 12d.

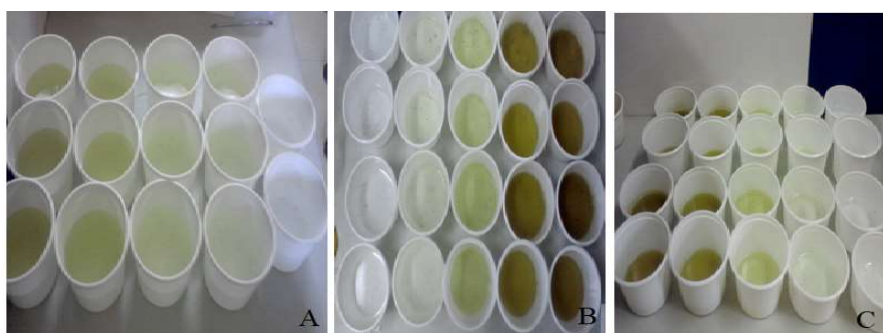


Figure No. 8: Preliminary Screening and toxicity studies for larvicidal activity of PEE of a) *Cleome viscosa*, b) *Artocarpus lakoocha* Leaf, c) *Artocarpus lakoocha* Fruit

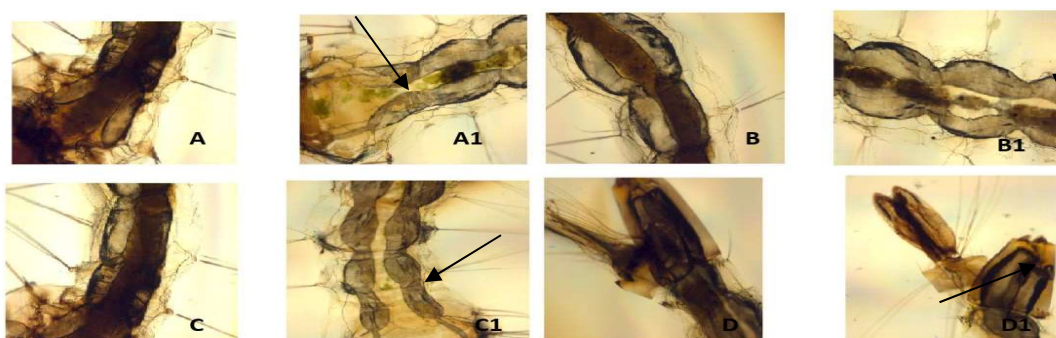


Figure No. 9: Larvicidal effects of CHE of *Cleome viscosa* on *Aedes aegypti*; A, B, C, D- control; A1, B1, C1- effects of CHE at 100 ppm on anterior and posterior midgut; D1- effects of CHE on anal respiratory gills

Table No. 12a: Preliminary larvicidal bioassay of *Cleome viscose*

Species	E	LC ₅₀ (ppm)	LC ₉₀ (ppm)	χ^2	Limits for LC ₅₀	
					LCL	UCL
<i>Anopheles stephensi</i>	PEE	2259.33	4697.78	11.98	2180.93	2337.73
	CHE	141.99	649.56	3.54	90.35	474.22
	ETE	1617.76	4754.94	9.12	1494.73	1752.52
	WE	4422.34	6788.45	51.43	3985.67	6932.77
	AZ	058.58	086.66	028.48	048.78	128.62
<i>Culex quinquefasciatus</i>	PEE	736.29	4080.48	8.99	327.16	1022.76
	CHE	035.68	368.75	1.22	020.73	052.85
	ETE	1033.62	3694.99	6.27	943.55	1108.04
	WE	3345.65	6298.45	023.65	2977.56	3647.69
	AZ	047.43	085.53	043.45	032.75	096.52
<i>Aedes aegypti</i>	PEE	898.31	3083.62	25.07	468.99	1326.06
	CHE	386.32	937.933	38.46	98.17	590.43
	ETE	1777.73	4020.70	4.44	1487.50	1991.82
	WE	3802.56	6890.51	28.38	3598.46	6946.64
	AZ	110.36	146.34	27.33	97.65	145.47
E- extract, PEE- petroleum ether extract, CHE- chloroform extract, ETE- ethanolic extract, WE- Aqueous extract, AZ- standard azadirachtin, LC ₅₀ - Lethal concentration at which 50% larvae are dead, LC ₉₀ -Lethal concentration at which 90% larvae are dead, χ^2 - Chi-square values, LCL- Lower confidence limit, UCL-Upper confidence limit, (p ≤0.05)						

The order of activity for *Cleome viscose* were CHE> ETE> PEE> WE which indicates the correlation of phytoconstituents such as terpenoids, steroids, and alkaloids to induce larvicidal effects as higher mortality.

ALL: In preliminary screening, PEE of both ALL and ALF had a significant larvicidal activity with LC₅₀ of 241.362, 624.885, 1162.867 ppm, and 1180.953 of, of 1286.694 and 1398.695 ppm on *Anopheles stephensi*, *Culex quinquefasciatus*, *Aedes aegypti* respectively (Table No. 12b, 12c). PEE of ALL and ALF were chosen for toxicity study, PEE of ALF had a better activity with LC₅₀ of 572.883, 1170.414, 1327.017 than the PEE of ALF with LC₅₀ of 1169.01, 1297.91, 1452.65 ppm on *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* (Table No. 12d).

The order of activity of the successive solvent extracts of ALL was PEE> CHE> ETE> PEE> WE and for ALF was PEE> ETE> CHE>WE. The difference in the order of activity may be due to differences in the qualitative and quantitative composition of

Table No. 12b: Preliminary larvicidal bioassay of *Artocarpus lakoocha* Leaf

Mosquito species	E	LC ₅₀ (ppm)	LC ₉₀ (ppm)	χ^2	Limits for LC ₅₀	
					LCL	UCL
<i>Anopheles stephensi</i>	PEE	241.36	2714.44	006.98	177.41	312.34
	CHE	732.88	4467.56	004.40	349.41	1199.61
	ETE	1022.07	1549.27	001.48	932.76	1106.46
	AZ	058.58	086.66	028.48	048.78	128.62
<i>Culex quinquefasciatus</i>	PEE	624.88	2994.99	68.45	268.78	1452.73
	CHE	1231.36	5339.85	16.67	470.24	3224.44
	ETE	1121.83	4675.60	35.83	539.22	2046.18
	AZ	047.43	085.53	043.45	032.75	096.52
<i>Aedes aegypti</i>	PEE	1162.86	1548.82	2.65	1130.85	1191.48
	CHE	1337.95	2377.36	01.16	1217.05	1461.99
	ETE	3082.68	5613.25	6.04	2922.43	3253.95
	AZ	110.36	146.34	27.33	97.65	145.47
LC₅₀ values >5000ppm Aqueous extracts were ineffective						
E- extract, PEE- petroleum ether extract, CHE- chloroform extract, ETE- ethanolic extract, WE- Aqueous extract, AZ- standard azadirachtin, LC ₅₀ - Lethal concentration at which 50% larvae are dead, LC ₉₀ -Lethal concentration at which 90% larvae are dead, χ^2 - Chi-square values, LCL- Lower confidence limit, UCL-Upper confidence limit, (p ≤0.05)						

phytoconstituents in leaves and fruits. Steroids, terpenoids, and flavanoids were identified in the CHE which may have induced mortality in the mosquito larvae. Petroleum ether extract also had similar phytoconstituents, however, the presence of additional phytochemicals such as triterpenoids and glycosides in CHE may have induced an additive or potentiation effect that may have resulted in higher mortality and

hence lower LC₅₀ values of CHE extract compared to the PEE extract as represented by study data in Table No. 12c.

Table No. 12c: Preliminary larvicidal bioassay of *Artocarpus lakoocha* Fruit

Mosquito species	E	LC ₅₀ (ppm)	LC ₉₀ (ppm)	χ^2	Limits for LC ₅₀	
					LCL	UCL
<i>Anopheles stephensi</i>	PEE	1180.95	1359.12	111.06	1145.55	1223.74
	ETE	1503.74	1850.44	8.80	1483.79	1525.36
	AZ	058.58	086.66	028.48	048.78	128.62
<i>Culex quinquefasciatus</i>	PEE	1286.69	1549.52	114.62	1232.16	1341.00
	ETE	1650.81	1940.66	35.25	1592.75	1717.01
	AZ	047.43	085.53	043.45	032.75	096.52
<i>Aedes aegypti</i>	PEE	1398.69	1658.52	001.62	1322.16	1356.00
	ETE	1944.32	2615.15	003.72	1738.42	2179.64
	AZ	110.36	146.34	027.33	097.65	145.47
LC ₅₀ values > 5000ppm, chloroform and aqueous extracts were ineffective						
E- extract, PEE- petroleum ether extract, CHE- chloroform extract, ETE- ethanolic extract, WE- Aqueous extract, AZ- standard azadirachtin, LC ₅₀ - Lethal concentration at which 50% larvae are dead, LC ₉₀ -Lethal concentration at which 90% larvae are dead, χ^2 - Chi-square values, LCL- Lower confidence limit, UCL-Upper confidence limit, (p ≤0.05)						

Toxicity Larvicidal Bioassay:

Among the plant parts of AL, the leaf extracts had better larvicidal activity compared to the fruit extracts. On comparison of CVA with ALL, CVA had better promising larvicidal activity with lower LC₅₀ values. The order of activity of the active extracts were CHE of CVA> PEE of ALL> PEE of ALF. Similarly, the order of sensitivity of the mosquito larvae to all the extracts were *Anopheles stephensi*> *Culex quinquefasciatus* > *Aedes aegypti*.

Table No. 12d: Statistical data of Toxicity studies of active extracts

Mosquito species		LC ₅₀ (ppm)	LC ₉₀ (ppm)	χ^2	Limits for LC ₅₀	
					LCL	UCL
CHE of CVA	<i>Ans</i>	427.67	555.85	1.10	409.44	442.94
	<i>Cxq</i>	455.92	490.92	0.23	445.50	462.63
	<i>Aea</i>	634.44	864.50	2.99	579.75	675.41
PEE of ALL	<i>Ans</i>	572.88	621.52	0.194	561.03	581.06
	<i>Cxq</i>	1170.41	1204.01	0.048	1142.13	1183.02
	<i>Aea</i>	1327.01	1533.15	0.31	1234.42	1379.97
PEE of ALF	<i>Ans</i>	1169.01	1229.31	37.82	1163.12	1174.62
	<i>Cxq</i>	1297.91	1384.39	3.01	1262.53	1392.84
	<i>Aea</i>	1452.65	1664.66	37.17	1431.36	1473.89
Ans- <i>Anopheles stephensi</i> , Cxq- <i>Culex quinquefasciatus</i> ; Aea- <i>Aedes aegypti</i> ; LC₅₀- Lethal concentration at which 50% larvae are dead, LC₉₀- Lethal concentration at which 90% larvae are dead, χ^2- Chi-square values, LCL- Lower confidence limit, UCL- Upper confidence limit, ($p \leq 0.05$)						

4.4: Oviposition Deterrence Activity

CVA: The ER₅₀ values of 18.933, 13.353, 151.44, and 1680.890 ppm were recorded for PEE, CHE, ETE, and WE on *Anopheles stephensi* with similar results for *Culex* and *Aedes* are presented in Table No. 13. 100% deterrence was at 100, 50, 300, and greater than 1000ppm for PEE, CHE, ETE, and WE, and hence, CHE of CVA was the most effective Oviposition deterrent compared to other tested extracts.

ALL: Out of the extracts screened, only the PEE showed effective deterrence with ER₅₀ of 104.956, 113.991, and 214.402 ppm for PEE for *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* respectively (Table No. 13). With ALF, the PEE had ER₅₀ of 353.318, 908.989, and 772.481 ppm for *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* respectively. 100% deterrence was 700, 1100, and 2000 ppm for three species. Other solvent extracts viz., CHE, ETE and WE displayed no or zero deterrence with no effects on oviposition (Table No. 13).

Table No. 13: Oviposition Deterrence Activity of *Cleome viscosa* and *Artocarpus lakoocha* Leaf

Mosquito species	E	<i>Cleome viscosa</i>					<i>Artocarpus lakoocha</i> Leaf				
		ER ₅₀ (ppm)	ER ₉₀ (ppm)	χ^2	Limits		ER ₅₀	ER ₉₀	χ^2	Limits	
					LCL	UCL				LCL	UCL
<i>Anopheles stephensi</i>	PEE	18.93	53.97	80.66	15.65	22.71	104.95	186.28	17.88	94.71	116.02
	CHE	13.35	34.80	73.80	10.47	016.848	ER ₅₀ values >5000ppm, chloroform extract ineffective				
	ETE	151.44	326.71	199.06	130.05	176.94	226.66	406.11	34.39	209.25	245.16
	WE	1680.89	2450.90	120.56	1459.89	1763.46	ER ₅₀ values >5000ppm, aqueous extract ineffective				
	AZ	12.45	16.56	14.78	9.78	13.79	12.45	16.56	14.78	9.78	13.79
<i>Culex quinquefasciatus</i>	PEE	31.59	089.73	214.67	025.47	038.99	113.99	209.88	17.90	107.97	120.02
	CHE	23.92	63.30	191.63	19.00	29.93	ER ₅₀ values >5000ppm, chloroform extract ineffective				
	ETE	311.24	792.38	220.81	266.47	363.46	274.51	477.29	28.56	256.40	293.55
	WE	1568.45	2244.56	32.68	1512.22	1695.86	ER ₅₀ values >5000ppm, aqueous extract ineffective				
	AZ	13.45	14.67	16.76	12.84	15.67	13.45	14.67	16.76	12.84	15.674
<i>Aedes aegypti</i>	PEE	40.01	113.81	185.08	33.60	47.52	214.40	434.81	19.37	203.95	224.91
	CHE	28.63	84.54	116.59	24.13	33.80	ER ₅₀ values >5000ppm, chloroform extract ineffective				
	ETE	760.82	1702.27	38.38	708.47	815.48	386.84	721.49	79.88	354.87	421.07
	WE	1898.85	2360.64	45.74	1683.65	1975.86	ER ₅₀ values >5000ppm, aqueous extract ineffective				
	AZ	15.69	18.82	19.99	13.78	19.83	15.69	18.82	19.99	13.78	19.83
E- extract, PEE- petroleum ether, CHE- chloroform extract, ETE- ethanolic, WE- Aqueous, AZ- standard azadirachtin, ER ₅₀ - Effective repellence concentration at which 50% mosquito are repelled, ER ₉₀ - Effective repellence concentration at which 90% mosquito are repelled, χ^2 - Chi-square values, LCL- Lower confidence limit, UCL-Upper confidence limit, (p ≤0.05)											

The order of activity of *Artocarpus lakoocha* Leaf was PEE> CHE> ETE> PEE> WE and for ALF was PEE> ETE> CHE>WE. The difference in the order of activity may be due to differences in the qualitative and quantitative composition of phytoconstituents in leaves and fruits. Steroids, terpenoids, and flavonoids were identified in the CHE which may have induced mortality in the mosquito larvae. Petroleum ether extract also had similar phytoconstituents, however, the presence of additional phytochemicals such as triterpenoids and glycosides in CHE may have induced an additive or potentiation effect that may have resulted in higher mortality and hence lower ER₅₀ values of CHE extract compared to the PEE extract.

Table No. 14: Oviposition Deterrence Activity of *Artocarpus lakoocha* Fruits

Mosquito species	E	ER ₅₀	ER ₉₀	χ^2	Limits for ER ₅₀	
					LCL	UCL
<i>Anopheles stephensi</i>	PEE	353.31	834.67	49.19	305.64	404.70
	AZ	12.45	16.56	14.78	9.78	13.79
<i>Culex quinquefasciatus</i>	PEE	908.98	1636.23	17.08	835.12	990.49
	AZ	13.45	14.67	16.76	12.84	15.67
<i>Aedes aegypti</i>	PEE	772.48	1713.99	6.72	724.93	823.15
	AZ	15.69	18.82	19.99	13.78	19.83
chloroform, ethanolic, and aqueous extracts ER ₅₀ values >5000ppm						
E- extract, PEE- petroleum ether extract, AZ- standard azadirachtin, ER ₅₀ - Effective repellents concentration at which 50% mosquitoes are repelled, ER ₉₀ - Effective repellents concentration at which 90% mosquitoes are repelled, χ^2 - Chi-square values, LCL- Lower confidence limit, UCL- Upper confidence limit, (p ≤0.05)						

4.5: Juvenile hormone-mimicking / Insect growth regulatory (IGR) activity

The JH activity was performed at concentrations below the LC₅₀ values of larvicidal bioassay with potential of extracts on morphological abnormalities in larvae pupae and adults. For CHE of CVA, EI₅₀ values of 16.065, 6.714 106.422 ppm for *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti* were recorded. With PEE of

ALL, EI_{50} values of 163.01, 185.90, 190.76 ppm for *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti* were found. JH was significant and higher than the control and lower than the standard. Water extracts were least effective with EI_{50} values greater than 3000ppm for all species (Table No. 15). In CVA, among the PEE and ETE, the PEE had better activity with low EI_{50} values, and its larval period increased to 6-8 days compared with that of the control Figure No. 10. At 10- 50 ppm, mortality as un-pigmented pupae was significant in PEE and was more effective than the ethanolic extracts.

With the fruits of AL, PEE was effective at EI_{50} of 172.667, 275.975, 278.247 ppm and ETE with EI_{50} of 502.984, 502.98, 1563.650 ppm (Table No. 15) while CHE showed no inhibition on *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* respectively.

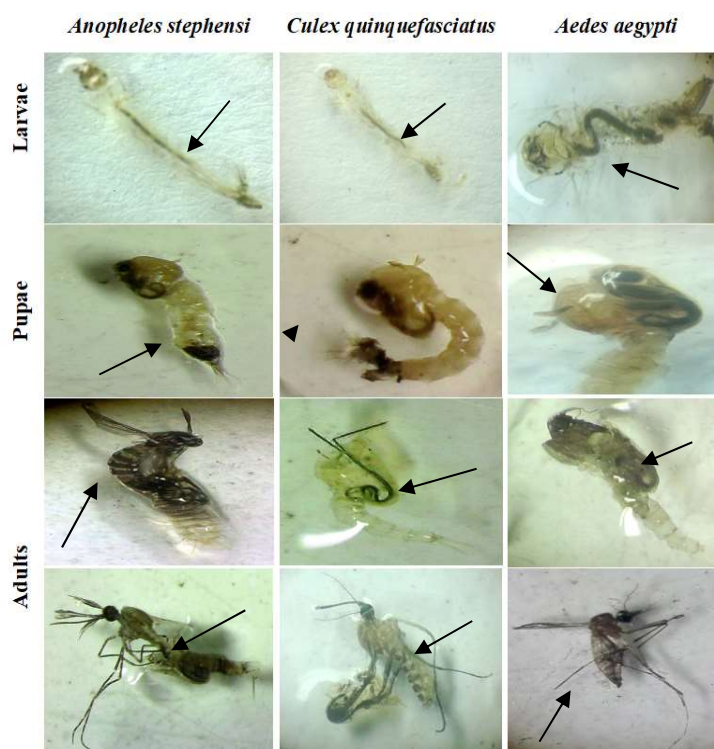


Figure No. 10: JHA activity of CHE of *Cleome viscosa* at 50 ppm indicating hormone related growth effects on *Aedes aegypti* larvae and mosquitoes

Table No. 15: IGR/JHA of *Cleome viscosa* and *Artocarpus lakoocha* Leaf

Plant	<i>Cleome viscosa</i>					<i>Artocarpus lakoocha</i> leaf					ALF				
E	EI ₅₀	EI ₉₀	χ^2	Limits for EI ₅₀		EI ₅₀	EI ₉₀	χ^2	Limits for EI ₅₀		EI ₅₀	EI ₉₀	χ^2	Limits for EI ₅₀	
				LCL	UCL				LCL	UCL				LCL	UCL
<i>Anopheles stephensi</i>															
PEE	24.89	143.50	32.95	12.51	47.68	163.01	307.90	5.78	113.91	231.25	172.66	332.65	1.17	90.96	262.19
CHE	16.06	103.47	30.36	7.750	31.47	468.24	1603.70	7.44	422.94	518.13	EI ₅₀ values >5000ppm, ineffective				
ETE	194.7	1154.32	50.76	115.1	327.14	714.12	285.20	26.81	413.77	1273.69	502.98	798.59	12.58	490.29	516.14
AZ	3.64	6.66	18.76	2.458	4.68	3.646	6.664	18.76	2.458	4.682	3.64	6.66	18.76	2.458	4.68
<i>Culex quinquefasciatus</i>															
PEE	13.18	99.23	7.773	1.023	33.48	185.90	654.79	3.56	116.5	289.77	275.97	479.95	19.94	249.12	302.63
CHE	6.714	10.79	1.17	5.837	7.218	575.13	869.10	65.79	402.34	821.38	EI ₅₀ values >5000ppm, ineffective				
ETE	532.1	797.01	9.838	433.1	652.19	473.15	603.75	9.92	439.12	510.51	502.98	798.59	12.58	490.29	516.14
AZ	4.562	7.773	23.54	3.558	6.728	4.562	7.773	23.54	3.558	6.728	3.64	6.66	18.76	2.458	4.68
<i>Aedes aegypti</i>															
PEE	165.2	353.66	19.94	50.60	205.31	190.7	322.39	8.18	119.78	147.95	278.24	465.41	1.04	248.37	309.54
CHE	106.4	208.82	1.044	104.7	127.15	486.15	808.32	11.15	133.40	1770.85	EI ₅₀ values >5000ppm, ineffective				
ETE	575.7	910.68	2.428	544.9	614.52	1944.3	5615.15	31.72	1738.4	2179.64	1563.6	1697.9	12.79	1364.5	1877.5
AZ	7.45	9.44	33.89	5.62	7.87	7.45	9.44	33.89	5.62	7.87	7.45	9.44	33.89	5.62	7.87
	EI ₅₀ values > 5000ppm, aqueous extract was ineffective					EI ₅₀ values >> 3000ppm aqueous extract was ineffective					EI ₅₀ values >> 3000ppm chloroform & Aqueous extract was ineffective				
E- extract, PEE- petroleum ether extract, CHE- chloroform extract, ETE- ethanolic extract, WE- Aqueous extract, AZ- standard azadirachtin, ER ₅₀ - Effective repellence concentration at which 50% mosquito are repelled, ER ₉₀ - Effective repellence concentration at which 90% mosquito are repelled, χ^2 - Chi-square values, LCL- Lower confidence limit, UCL-Upper confidence limit, (p ≤0.05)															

4.6: Bioactivity-Guided Isolation (BAGI) of Larvicidal Compounds

From the results of larvicidal bioassay, juvenile hormone-mimicking IGR activity, and oviposition deterrence activity, it was evident that **CHE of *Cleome viscosa*** and **PEE of *Artocarpus lakoocha* Leaf** was the most effective and significant activity as a pesticidal agent.

4.6.1: Bioactivity-Guided Isolation of CHE of *Cleome viscosa*

Bioactivity-guided isolation of CHE was carried out on an open column of silica gel and subsequent elution with various proportions of solvent petroleum ether and ethyl acetate to get fifteen fractions (F1-F52). The recorded weights, nature of fractions, and TLC patterns are as in Table No. 16.

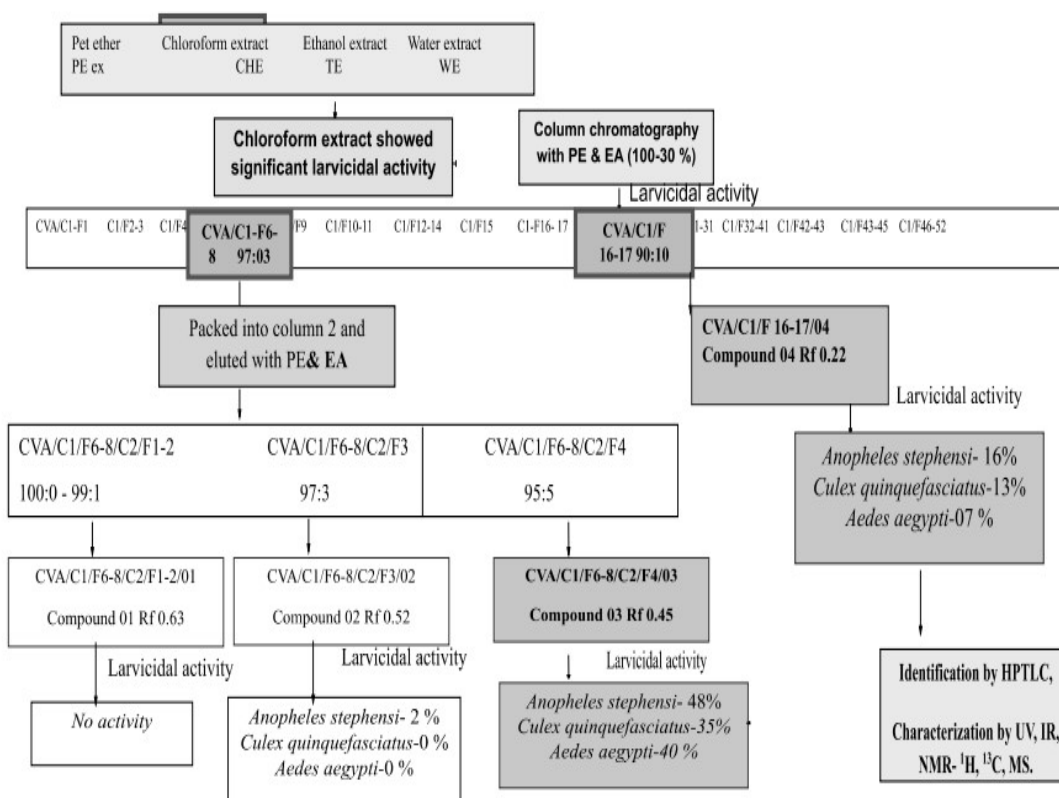


Figure No. 11: Bioactivity Guided Isolation of Chloroform extract of *Cleome viscosa*

Table No. 16: Column chromatography of Chloroform extract of *Cleome viscosa*

Sl. No.	PE: EA	Fraction	Weight (mg)	R _f values in T: EA
1	100:0	CVA/C1/F1	450	No separation
2	99:01	CVA/C1/F2-3	180	0.91
3	99:01	CVA/C1/F4-5	338	0.78, 0.73,0.64,0.63
4	97:03	CVA/C1/F6-8	474	0.63, 0.52, 0.45
5	95:05	CVA/C1/F9	294	0.45
6	95:05	CVA/C1/F10-11	270	0.54, 0.50,0.45
7	95:05	CVA/C1/F12-14	90	0.42
8	90:10	CVA/C1/F 15	45	0.42, 0.22
9	90:10	CVA/C1/F16-17	112	0.22
10	90:10	CVA/C1/F 18-20	68	0.22,0.17
11	80:20	CVA/C1/F21-31	248	0.17,0.08
12	80:20	CVA/C1/F32-41	135	0.17,0.08
13	70:30	CVA/C1/F42-43	69	0.17,0.08
14	60:40	CVA/C1/F44-45	114	0.04
15	60:40	CVA/C1/F46-52	114	No separation
PE- Petroleum ether, EA- Ethyl acetate, T- Toluene; R _f values after derivatization with VS reagent				

The fractions were subjected to larvicidal activity at 100 ppm. Fraction **CVA/C1/F6-8** had a significant larvicidal activity of 82, 100, and 100% on *Anopheles stephensi*, 84, 100, 100% mortality on *Culex quinquefasciatus*, and 46, 88,100% mortality on *Aedes aegypti* at 24, 48, 72 hrs respectively. Fraction CVA/C1/F16-17 displayed a significant larvicidal activity with 60, 78, and 90% mortality on *Anopheles stephensi*, 48, 56, 76 % mortality on *Culex quinquefasciatus* and 28, 45, 62 % mortality on *Aedes aegypti* at 24, 48, and 72 hrs respectively (Table No. 17). The sensitivity of the mosquito species were *Anopheles stephensi* > *Culex quinquefasciatus* > *Aedes aegypti* for all time intervals of 24, 48 and 72 hrs. The order of activity of the successive solvent extracts of CVA were

CHE> ETE> PEE> WE which indicates the correlation of phytoconstituents such as terpenoids, steroids, and alkaloids to induce larvicidal effects as higher mortality.

Table No. 17: Percentage larval Mortality of column fractions of Chloroform extract of *Cleome viscosa*

Sl. No.	Column/ Fraction	% Mortality								
		<i>Anopheles stephensi</i>			<i>Culex quinquefasciatus</i>			<i>Aedes aegypti</i>		
	Days	1	2	3	1	2	3	1	2	3
1	CVA/C1/F1	00	00	00	00	00	00	00	00	00
2	CVA/C1/F 2-3	00	00	00	00	00	00	00	00	00
3	CVA/C1/F4-5	00	10	27	00	06	12	00	02	10
4	CVA/C1/F6-8	82	100	100	84	100	100	46	88	100
5	CVA/C1/F9	03	10	18	00	13	23	00	08	15
6	CVA/C1/F10-11	02	08	20	00	03	15	00	03	12
7	CVA/C1/F12-14	00	00	02	00	01	06	00	00	00
8	CVA/C1/F 15	00	00	04	00	00	01	00	00	00
9	CVA/C1/F16-17	60	78	90	48	56	76	28	45	62
10	CVA/C1/F18-20	05	20	34	02	18	26	00	12	18
11	CVA/C1/F21-31	00	02	06	00	01	04	00	00	01
12	CVA/C1/F32-41	00	00	00	00	00	00	00	00	00
13	CVA/C1/F42-43	00	00	04	00	00	02	00	00	00
14	CVA/C1/F44-45	00	00	00	00	00	00	00	00	00
15	CVA/C1/F46-52	00	00	00	00	00	00	00	00	00
CVA: <i>Cleome viscosa</i> Linn.; C1: column 1; Fractions collected: F1-52; fractions 1-52										

The fraction **CVA/C1/F6-8** was re-purified by separation on a silica gel flash column and eluted with petroleum ether: ethyl acetate resulted in three fractions CVA/C1/F6-8/C2/F1 to F3 with three isolated compounds with Rf values of 0.63, 0.52, 0.45. The compounds were designated as **CVA/C1/F6-8/C2/F1/comp-01**, **CVA/C1/F6-8/C2/F2/comp-02** and **CVA/C1/F6-8/C2/F3/comp-03** with 0.45, 0.25, and 0.41 % w/w yield with respect to the fraction collected. 10-50 ppm of isolated compounds were subjected to larvicidal activity. The percentage mortality was noted hourly for the first

6 hrs and then for every 6 hrs, up to 72 hrs (Table No. 18). CVA/C1/F6-8/C2/F4/comp-01 and CVA/C1/F16-17/comp-02 were inactive as larvicidal agents. While compounds **CVA/C1/F6-8/C2/F4/comp-03** and **CVA/C1/F16-17/comp-04** had a significant larvicidal activity with its LC₅₀ values below 60 ppm (Table No. 18). Figure No. 11 depicts the overall isolation of larvicidal compounds from CHE.

Table No. 18: Larvicidal activity of isolated compounds from Chloroform extract of *Cleome viscosa*

Isolated compounds	Mosquito Species	LC ₅₀ (ppm)	LC ₉₀ (ppm)	χ^2	Limits for LC ₅₀	
					LCL	UCL
CVA/C1/F6-8/C2/F4/ 03	<i>Anopheles stephensi</i>	7.59	13.24	19.99	6.576	8.74
	<i>Culex quinquefasciatus</i>	7.34	14.86	35.03	5.857	9.19
	<i>Aedes aegypti</i>	13.21	19.24	3.75	12.750	13.68
CVA/C1/F16-17/04	<i>Anopheles stephensi</i>	27.01	40.87	27.99	26.45	27.57
	<i>Culex quinquefasciatus</i>	59.31	94.88	12.71	56.97	61.76
	<i>Aedes aegypti</i>	37.28	56.88	14.20	36.17	36.17
CVA/C1/F6-8/C2/F4/01 & CVA/C1/F6-8/C2/F4/02 were inactive at 10-50 ppm						

4.6.2: Bioactivity-Guided Isolation of Petroleum ether extract of *Artocarpus lakoocha* Leaf

Based on the results of larvicidal bioassay, JH activity, and oviposition deterrence, the effective PEE extract of leaves of *AL*, was subjected to *BAGI* of larvicidal compounds. From the column chromatography, 13 fractions (F1-F13) were pooled, concentrated, and dried. The recorded weights, nature of fractions, and TLC patterns are in Table No. 19. All 13 fractions, were subjected to larvicidal activity at 100 ppm (Table No. 20a) Fraction ALL/C1/F4-5 exhibited maximum activity of 60, 100, and 100% on *Anopheles stephensi*; 62, 100, and 100% on *Culex quinquefasciatus* and 67, 100, and 100% on *Aedes aegypti* respectively at 24, 48, and 72hr (Table No. 20a). The order sensitivity

of the mosquito species was *Anopheles stephensi* > *Culex quinquefasciatus* > *Aedes aegypti* for all time intervals of 24, 48 and 72 hrs.

Table No. 19: Column chromatography of Petroleum ether extract of *Artocarpus lakoocha* Leaf

Sl. No.	PE: EA	Fraction	Weight (mg)	R _f T: EA (8:2)
1	95: 5	ALL/C1/F1	1700	0.04,0.09
2	90: 10	ALL/C1/F2	1280	0.04,0.09,0.32
3	80: 20	ALL/C1/F3	550	0.45, 0.56
4	70:30	ALL/C1/F4	1650	0.56
5	60: 40	ALL/C1/F5	875	0.56, 0.64, 0.73
6	55:45	ALL/C1/F6	854	0.78
7	50:50	ALL/C1/F7	1925	0.82, 0.86, 0.9
8	40: 60	ALL/C1/F8	825	0.86, 0.9
9	30: 70	ALL/C1/F9	525	0.92, 0.94
10	20: 80	ALL/C1/F10	825	0.92, 0.94
11	10: 90	ALL/C1/F11-12	325	0.96, 0.98
12	0: 100	ALL/C1/F13	10	0.98
PE- Petroleum ether, EA- Ethyl acetate, T- toluene; R _f values after derivatization with Vanillin Sulphuric acid reagent; ALL- <i>Artocarpus lakoocha</i> Roxb., leaves; C1- column 1; Fractions F1-13. % yield - % w/w of extract				

Three sub fractions i.e., **ALL/C1/F4-5/C2/F1-3** was obtained by the re-chromatography of ALL/C1/F4-5. Three isolated compounds were designated as **ALL/C1/F4-5/C2/F1/Comp-01**, **ALL/C1/F4-5/C2/F2/Comp-02**, **ALL/C1/F4-5/C2/F3/comp-03** and subjected to larvicidal activity. The LC₅₀ values of isolated compounds are as in Table No. 20b.

Table No. 20a: Percentage mortality of larvicidal activity of column fractions of petroleum ether extract of *Artocarpus lakoocha* Fruit

Sl. No.	Fraction	% Mortality								
		<i>Anopheles stephensi</i>			<i>Culex quinquefasciatus</i>			<i>Aedes aegypti</i>		
	Days	1	2	3	1	2	3	1	2	3
1	ALL/C1/F1	00	00	01	00	00	00	00	00	00
2	ALL/C1/F2	00	12	20	01	16	19	00	08	15
3	ALL/C1/F3	03	18	35	02	20	30	00	16	26
4	ALL/C1/F4	00	00	00	00	00	00	00	00	00
5	ALL/C1/F5	64	92	100	62	90	100	57	92	98
6	ALL/C1/F6	00	00	00	00	00	00	00	00	00
7	ALL/C1/F7	00	00	00	00	00	00	00	00	00
8	ALL/C1/F8	00	00	00	00	00	00	00	00	00
9	ALL/C1/F9-10	00	00	00	00	00	00	00	00	00
10	ALL/C1/F11-12	01	01	02	00	01	01	00	00	01
11	ALL/C1/F13	01	01	01	00	01	01	00	00	01
ALL- <i>Artocarpus lakoocha</i> Roxb., leaves; C1- column 1; Fractions F1-1										

Figure No. 12 depicts the overall isolation of larvicidal compounds from PEE of ALL. ALL/C1/ F5/C2/ F3/comp-02 had no larvicidal activity. **ALL/C1/F4-5/C2/F1/Comp-01** and **ALL/C1/F4-5/C2/F2/Comp-02** had a significant larvicidal activity with LC₅₀ values less than 80 and 190 ppm respectively with sensitivity of *Anopheles stephensi* > *Culex quinquefasciatus* > *Aedes aegypti*, while ALL/C1/F4-5/C2/F3/Comp-03 was inactive as a larvicidal agent.

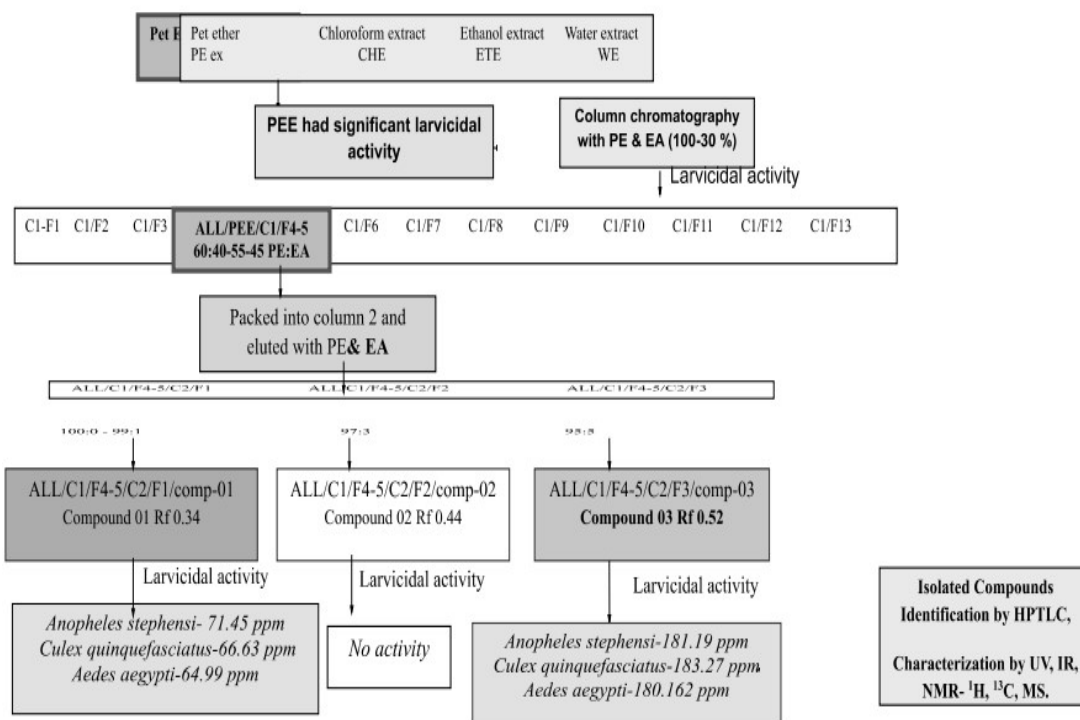


Figure No. 12: Bioactivity Guided Isolation of petroleum ether extract of *Artocarpus lakoocha* Leaf

Table No. 20b: Larvicidal activity of isolated compounds from petroleum ether extract of *Artocarpus lakoocha* Leaf

Isolated compound	Mosq. species	LC ₅₀ (ppm)	LC ₉₀ (ppm)	χ^2	Limits for LC ₅₀	
					LCL	UCL
ALL/C1/F5/C2/ F1-2/comp- 01	<i>Ans</i>	71.45	115.56	0.60	77.20	250.39
	<i>Cxq</i>	66.63	101.65	0.29	71.17	178.82
	<i>Aea</i>	64.99	100.18	0.32	68.46	158.21
ALL/C1/F5/C2 /F4-6/comp-03	<i>Ans</i>	181.19	219.65	39.90	163.14	340.89
	<i>Cxq</i>	183.27	112.96	36.76	172.16	194.38
	<i>Aea</i>	180.16	207.56	4.95	167.25	194.24
ALL/C1/ F5/C2/ F3/comp-02—no larvicidal activity						
C1- column, F- fractions, LC ₅₀ - Lethal concentration at which 50% mosquitoes are repelled, LC ₉₀ - Lethal concentration at which 90% mosquitoes are repelled, χ^2 - Chi-square values, LCL- Lower confidence limit, UCL-Upper confidence limit, (p ≤0.05)						

4.7: Characterization of extracts & isolated Compounds

Table No. 21: Characterization of Active Extracts (Refer Annexure I)

Spectra	Chloroform extract of <i>Cleome viscosa</i>	petroleum ether extract of <i>Artocarpus lakoocha</i> Leaf
UV (λ_{max})	219, 235, and 359 nm	238nm, 256nm, 359nm
IR (cm^{-1})	C-C: 506.44; Ar-H: 645.85, 966.51; C-N: 1294.77, N=N: 1348.56, C=O stretch: 1988.46, 2344.42, 2656.99; Ar-H: 2977.64; Ar-H: 3362.34, 3486.96, 3596.58, 3600.32	C=O stretch: 1988.46, 2343.42, 2482.56, 2654.99; Ar-H: 2976.64; Ar-H: 3365.34, 3487.96, 3596.58, 3600.32, 3806.42
HPLC		
205nm	Rt (min) of 0.84, 1.26, 1.52, 1.78, 2.43, 9.16, 14.36 (juglone), 15.08, 15.28	2.80 (a-amyrin), 10.2, 11.8 (cycloartenol), 18.4 (resveratrol)
HPTLC		
WL	R _f values: 0.75 and 0.80	R _f values: 0.68, 0.74, 0.87
UV 254nm	R _f values: 0.03, 0.07, 0.12, 0.22, 0.33, 0.45, 0.52, 0.54, 0.68, 0.73, 0.85, 0.91	R _f values 0.02, 0.18, 0.36, 0.40, 0.49, 0.52, 0.68, 0.73, 0.74, 0.87
UV 366nm	R _f values: 0.75, 0.80	0.02, 0.06, 0.28, 0.36, 0.40, 0.52, 0.68, 0.74, 0.87, 0.92

4.7.2: Characterization of Isolated Compounds

CVA/C1/F6-8/C2/F4/comp-03: White needles-shaped crystals, $\text{C}_{10}\text{H}_{18}\text{NS}_2\text{O}_{10}$ by its HRESIMS at m/z 353.4 $[\text{M} - \text{H}]^-$ (calculated for $\text{C}_{10}\text{H}_{18}\text{NS}_2\text{O}_{10}$: 354.291). The UV spectrum had an absorption maximum of 227, 235 nm in ethanol. The IR spectrum implied for the presence of cyclic ring of CH with one /two substitution (743.67 cm^{-1}), strong S-NR₂ (111.85 cm^{-1} , 1339.45 cm^{-1}), strong -SO₂- O- (1210.15 cm^{-1}), -C-N=O (1210.15 cm^{-1} , 1274.18 cm^{-1} , 1631.81 cm^{-1}), tertiary amine (2468.03 , 2628.78 cm^{-1}), -C=O-NH (3159.98 cm^{-1}), H bridge of -OH (3539.03 cm^{-1}).

The ¹H NMR of compound 1 displayed the characteristic shift values of glucosinolates with δH values of 6.024(1H, s, $J = 3.45\text{Hz}$), 5.303(1H, s, $J = 1.439\text{Hz}$), 4.918 (1H, s, $J = 29.88\text{Hz}$), 3.866 (1H, s, $J = 16.215\text{Hz}$), 3.569(1H, s, $J = .8.53\text{Hz}$), 3.484 (1H,

s, $J = 4.19\text{Hz}$), 3.320 (1H, s, $J = 16.789\text{Hz}$), 3.272 (1H, s, $J = 24.592\text{Hz}$), 3.24 (1H, s, $J = 11.52\text{Hz}$), 3.19 (1H, s, $J = 4.76\text{Hz}$), 2.133 (1H, s, $J = 1501\text{Hz}$). The ^{13}C NMR spectra showed the signals of aliphatic alkenes, and alkene of isothiocyanate, with signals of sugar moiety- D- glucopyranose. The ^1H NMR and ^{13}C NMR displayed signals for sugar moiety D- glucopyranose - glucose [δH 4.85 (1H, s, $J = 7.4\text{ Hz}$), 3.86 (1H, dd, $J = 12.0, 2.3\text{ Hz}$), 3.69 (1H, dd, $J = 12.0, 5.9\text{ Hz}$), 3.41 (1H, t, $J = 9.1\text{Hz}$), 3.36-3.39 (3H, m), δC 101.6(CH), 78.2(CH), 77.8 (CH), 75.8(CH), 70.6(CH), 62.7(CH₂)]. The test of nitrogen, sulphur, and reducing sugars was positive. The test for glucosinolates was by placing the picrate paper test at the mouth of the test tube, and hydrolysis with dilute HCl turned the paper to reddish brown due to exposure to released HCN gas. A TLC confirmatory test in n-butanol: n-propanol: GAA: water (30:10: 10: 10) had a blue spot with an R_f value of 0.45 under UV 254nm. The HPTLC chromatogram of CVA/C1/F6-8/C2/F4/comp-03 in toluene: ethyl acetate, derivatized with Vanillin sulphuric acid, had an R_f of 0.45 compared to the peak 7 of CHE (Annexure-II). Compound CV/C1/F6-8/C2/F4/comp-03 was 1- S- [N-(sulonatoxy) but-3 -nimidoyl] -1 -thio- beta – D glucopyranose (Figure No. 13).

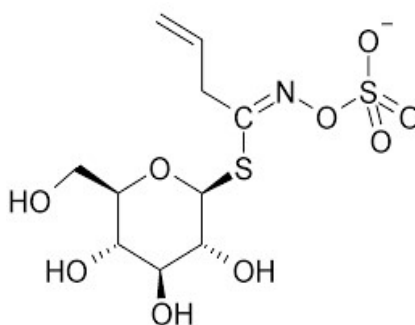


Figure No. 13: Structure of CVA/C1/F6-8/C2/F4/comp-03 - 1- S- [N-(sulonatoxy) but- 3 -nimidoyl] -1 -thio- beta – D glucopyranose or Sinigrin (allyl isothiocyanate)

CVA/C1/F16-17/comp-04: White needles-shaped crystals, $C_{16}H_{17}O_9$ by HRESIMS at m/z 357.8 $[M - H]^-$ (calculated for $C_{16}H_{17}O_9$: 356.142). UV spectrum showed adsorption maxima at 210 and 255nm in ethanol. The IR spectrum implied for the presence of aromatic ring 680.42 cm^{-1} , alkene of aromatic ring (898.38 cm^{-1}), Carbonyl group $-C=O$ (1229 cm^{-1}), $-C=C-$ with conjugated ring system ($1428.50, 1587.91, 1673.04\text{ cm}^{-1}$), aromatic $-OH$ (3051.72 cm^{-1}), H bridge of $-OH$ (3419.04 cm^{-1}).

The ^1H NMR of compound 1 displayed the characteristic shift values of naphthoquinones with δH values of 6.034 (1H, s, $J = 1.0\text{Hz}$), 5.318 (1H, s, $J = 0.54\text{Hz}$), 5.280 (1H, s, $J = 0.49\text{Hz}$), 4.92 (1H, s, $J = 1.07\text{ Hz}$), 3.92 (1H, s, $J = 1.10\text{ Hz}$), 3.92 (1H, s, $J = 1.47\text{Hz}$), 3.87 (1H, s, $J = \text{Hz}$), 3.32 (1H, s, $J = 0.82\text{ Hz}$), 3.30 (1H, s, $J = 0.34\text{Hz}$), 3.209 (1H, s, $J = 1.43\text{Hz}$). The ^{13}C NMR spectra showed the signal of aromatic alkene with carbonyl group in the ring system and D- D-glucopyranose. ^1H NMR and ^{13}C NMR displayed signals for sugar moiety D- glucopyranose - glucose [δH 4.85 (1H, s, $J = 7.4\text{ Hz}$), 3.86 (1H, dd, $J = 12.0, 2.3\text{ Hz}$), 3.69 (1H, dd, $J = 12.0, 5.9\text{ Hz}$), 3.41 (1H, t, $J = 9.1\text{Hz}$), 3.36-3.39 (3H, m), δC 101.6(CH), 78.2(CH), 77.8(CH), 75.8(CH), 70.6(CH), 62.7(CH₂)].

The test of nitrogen, sulphur, and reducing sugars was negative. Test for terpenoids- Salkowski and Liebermann Burchard tests, reducing sugars were positive and indicated terpenoids/ reducing sugar. TLC confirmatory test was performed in petroleum ether: ethyl acetate (7:3) and sprayed with 10% methanolic KOH. A yellowish-brown spot at the R_f value of 0.22 was found in UV 254nm. HPTLC of the isolated compound was in toluene: ethyl acetate and was derivatized with VS reagent had R_f of 0.22.

From the ^1H , ^{13}C NMR, the structure of compound CVA/C1/F16-17/04 was 5-hydroxy 1,4 naphthoquinone di-one- 6- beta – D glucopyranose (Figure No. 14).

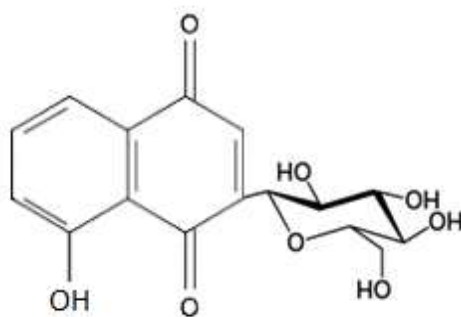


Figure No. 14: Structure of CVA/C1/F16-17/04- 5- hydroxy 1, 4 naphthoquinone di-one- 6- beta – D glucopyranose or Juglone-3-D- glucopyranose

ALL/C1/F4-5/C2/F1-2/comp-01: White rhombic crystals, $C_{30}H_{50}O$ by its HRESIMS at m/z 425.25 $[M - H]^-$ (calculated for $C_{30}H_{50}O$ 425.25). UV (methanol) λ_{max} (log ϵ) 316(0.246), 256 (0.874), 238 (0.203), 196(0.232) nm; MP. 122-124°C; solubility: petroleum ether, methanol, DMSO; TLC R_f -0.62 in PE: EA (8:2). The IR spectrum implied the presence of free OH of alcohol (4356, 4254, 3844 cm^{-1}), with OH stretching (3734 cm^{-1}), attachment of hydroxyl group to methylenic carbon $-CH_2-C=O$ (2714 cm^{-1}), $C=C$ in alkene side chain (2232, 1738, 1632 cm^{-1}), $CH=CH$ trans conformation (1556 cm^{-1}), free methyl bending anti syn deformation (1442 cm^{-1}) $CH=CH_2$ vinyl with CH out of plane (1275, 942 cm^{-1}), primary-OH in cyclic alcohol with $C=O$ stretch (1251 cm^{-1}), the six-member cyclic ring system of cyclic compounds (1021 cm^{-1}), disubstituted alkene CH out of the plane (839, 799 cm^{-1}).

The δH , δC values ^1H NMR and ^{13}C NMR spectra of isolated compound 1 displayed the characteristic shift values of the steroidal moiety. ^1H NMR displayed δH values of 6.5423 (1H, s, $J = 0.23$ Hz), 6.2985 (2H, s $J = 1.88$ Hz), 6.2546 (1Hs, $J = 1.42$ Hz), 5.3421 (1H, s, $J = 8.315$ Hz), 5.8823 (1Hs, $J = 10.789$ Hz), 1.2310 (1H, s, $J = 1.1592$ Hz), 0.9861 (1H,s, $J = 1.1623$ Hz), 0.9724 (1H, s, $J = 1.165$ Hz), 0.8231 (1H,s, $J = 1.316$ Hz). The ^{13}C NMR spectra showed the presence of δC 168.54, 136.21, 122.02(s,

-C=C-), 96.74 (s, C-O), 73.58(CH₂-CH₂), 58.95, 46.03 (s,-H₂C-O-), 39.62, 39.50, 36.38, (H₂C-C 2°), 26.24, 22.92, 19.10, 14.4, 12.2 (>CH-C- tertiary 3° methyl signals). The signals at 122.0 (s) and 136.0 (d) indicate characteristics of steroidal moiety. TLC analysis also confirmed that the compound was cycloartenol in n-hexane: ethyl acetate (5:1) gave an R_f of 0.34. The chemical analysis of compound 1 by the Salkowski test also indicated that the isolated compound is a steroidal molecule. From the literature survey, the isolated compound shows the physicochemical & spectral characteristics of “Cycloartenol” (Figure No. 15).

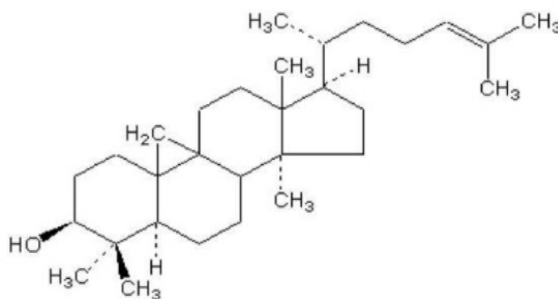


Figure No. 15: Structure of ALL/C1/F5/C2/F1-2/comp- 01: Cycloartenol (9 β,19-cyclo- 24-lanosten-3β-ol)

ALL/C1/F5/C2/F1-2/comp-02: White amorphous powder, UV spectrum showed adsorption maxima at 240 nm in methanol. C₂₆H₃₀O₄ by HRESIMS at m/z 404.6 [M - H]⁻ (calculated for C₂₆H₃₀O₄: 405.6). The IR spectrum implied for the presence of H bridge of -OH (3417 cm⁻¹), C-O bond of furan ring (1200 cm⁻¹), C=O Carbonyl group (1633 cm⁻¹), alkyl groups- C-H stretching (2965, 2920, 2855 cm⁻¹). ¹H-NMR: 6.86 (1H, d, *J* = 1.0), 6.29 (1H, d, *J* = 2.0), 6.49 (1H, d, *J* = 2.0), 6.80 (s), 3.47 (1H, d, *J* = 6.5), 5.65 (1H, d, *J* = 10.5), 2.07(m), 1.57(s), 1.64(s), 5.13 (1H, brt, *J* = 7.0). The ¹³C NMR spectra showed the signal with distinct peaks for the carbons of the benzyl group, methylene carbon connecting to the furan ring and carbon directly attached to the benzyl group. Benzyl carbon- 154.2 (s), 153.0(s), 103.1.0 (d), 112.9(s), 121.2(d),

157.7.4(s); Methylene carbon (CH₂)- 98.6(d), 131.9(s); β-carbons- 107.6 (d), 124.8(d), 25.9(q), 42.0(t), 25.9(q) 17.7(q). From the literature survey, the isolated compound shows the physicochemical & spectral characteristics of “2-aryl benzo furans: LakochinA” (Figure No. 16).

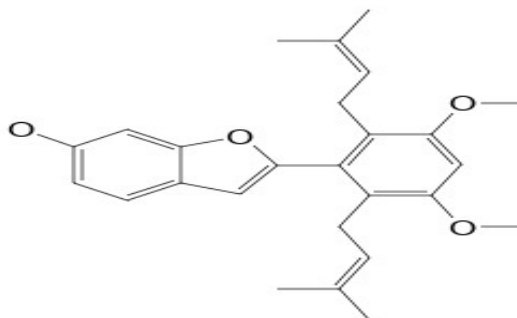


Figure No. 16: Structure of ALL/C1/F5/C2/F1-2/comp-02 2-arylbenzofurans: LakochinA

ALL/C1/F5/C2/F1-2/comp-03: Yellow to white amorphous powder; C₂₄H₂₆O₄ by HRESIMS at m/z 377.5445.2381 (calculated for C₂₄H₂₆O₄, 378.5). UV spectrum showed adsorption maxima at 210 and 234 nm in methanol. The IR spectrum implied for the presence of aromatic C-H stretching (3030 cm⁻¹), aliphatic C-H stretching (2966, 2924, 2854 cm⁻¹), strong C=C stretching peak from the furan ring between 1607, 1489, 1445 cm⁻¹), C-O stretching peak from the furan ring 1165 cm⁻¹). The ¹H NMR of compound 1 displayed the characteristic shift values of benzyl group's aromatic protons with δH values of 6.024 (1H, s, *J* = 1.0Hz), 5.328(1H, s, *J* = 0.54Hz), 5.27 (1H, s, *J* = 0.47Hz), 4.92 (1H, s, *J* = 1.12 Hz), furan ring protons 6.95 (1H, d, *J*=2.0Hz), 6.70(1H, s, *J* = 1.12 Hz), 3.45(1H, d, *J* = d, 6.5 Hz), methylene group of benzyl group- 5.17(1H, s, *J* = 6.5 Hz), 5.57(1H, d, *J* = 10 Hz). The ¹³C NMR spectra showed the signal with distinct peaks for the carbons of the benzyl group, methylene carbon connecting to the furan ring and carbon directly attached to the benzyl group. Benzyl

carbon- 154.4(s) 105.0 (d), 122.9(s), 121.2(d), 153.4(s), Methylene carbon (CH₂)- 98.2(d), 130.2(s), 152.3(s), β -carbons- 106.9(d), 25.7(q), 128.6(d), 26.2(q), 22.9(t), 124.2(d), 131.7(s), 17.6(q), 25.6(q). From the literature survey, the isolated compound shows the physicochemical & spectral characteristics of “Albafuran B” (Figure No. 17).

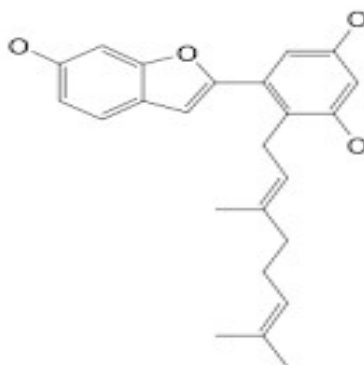


Figure No. 17: Structure of ALL/C1/F5/C2/F1-2/comp-03: Albafuran B

4.8: Effects on non-target organism: Guppy Fishes: *Poecilia reticulata*

The toxicity of CHE of CVA and PEE of *Artocarpus lakoocha* Leaf and its isolated compounds on *Poecilia reticulata*, *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* are as in Table No. 22a.

The LC₅₀ of 7165.682, 856.379, and 1194.165 ppm on non-target organism *Poecilia reticulata* were obtained for CHE of CVA. CVA/C1/F6-8/C2/F4/comp-03 and CVA/C1/F16-17/comp-04 respectively (Table No. 22a and Figure No. 18a).

The LC₅₀ of 1948.02, 858.58, 980.46 ppm on *Poecilia reticulata* were obtained for PEE of ALL, ALL/C1/F4-5/C2/F1-2/comp-01, ALL/C1 /F5/C2/F4-6/comp-03 respectively (Table No. 22a and Figure No. 18a).

Table No. 22a: LC₅₀ values of extracts and the isolated compounds on *Poecilia reticulata* - a non-target organism

Isolated compound	Mosquito Species	LC ₅₀ (ppm)	LC ₉₀ (ppm)	χ^2	Limits for LC ₅₀	
					LCL	UCL
CHE of <i>Cleome viscosa</i>	<i>Ans</i>	141.99	649.56	3.54	90.35	474.22
	<i>Cxq</i>	35.68	368.75	1.22	20.73	52.85
	<i>Aea</i>	386.32	937.93	38.46	98.17	590.43
	<i>Poecilia reticulata</i>	7165.68	2896.86	1.217	1346.1	1468.87
CVA/C1/F6-8/C2/F4/comp-03	<i>Ans</i>	7.59	13.24	19.99	6.57	8.74
	<i>Cxq</i>	7.34	14.86	35.03	5.85	9.19
	<i>Aea</i>	13.21	19.24	3.75	12.75	13.68
	<i>Poecilia reticulata</i>	856.37	1324.94	5.35	788.46	924.29
CVA/C1/F16-17/comp-04	<i>Ans</i>	27.01	40.87	27.99	26.45	27.57
	<i>Cxq</i>	59.31	94.88	12.71	56.97	61.76
	<i>Aea</i>	37.28	56.88	14.20	36.17	36.17
	<i>Poecilia reticulata</i>	1194.16	1902.44	4.71	1121.7	1266.56
PEE of ALL	<i>Ans</i>	241.36	2714.44	6.98	177.41	312.34
	<i>Cxq</i>	624.88	2994.99	68.45	268.78	1452.73
	<i>Aea</i>	1162.86	1548.82	2.65	1130.0	1191.48
	<i>Poecilia reticulata</i>	1948.02	1866.62	8.90	1844.7	1976.98
ALL/C1/F4-5/C2/F1-2/comp-01	<i>Ans</i>	71.45	115.56	0.60	77.20	250.39
	<i>Cxq</i>	66.63	101.65	0.29	71.17	178.82
	<i>Aea</i>	64.99	100.18	0.32	68.46	158.21
	<i>Poecilia reticulata</i>	858.58	1956.46	6.720	752.91	1166.72
ALL/C1/F5/C2/F4-6/comp-03	<i>Ans</i>	181.19	219.65	39.90	163.14	340.89
	<i>Cxq</i>	183.27	112.96	36.76	172.16	194.38
	<i>Aea</i>	180.16	207.56	4.959	167.25	194.24
	<i>Poecilia reticulata</i>	980.46	1588.84	2.672	954.82	1244.28
CVA- <i>Cleome viscosa</i> Linn., ALL- <i>Artocarpus lakoocha</i> Roxb., leaves; CHE- chloroform, PE- Petroleum ether, C1- column 1; C2- column 2; F- Fractions, comp- isolated compound						

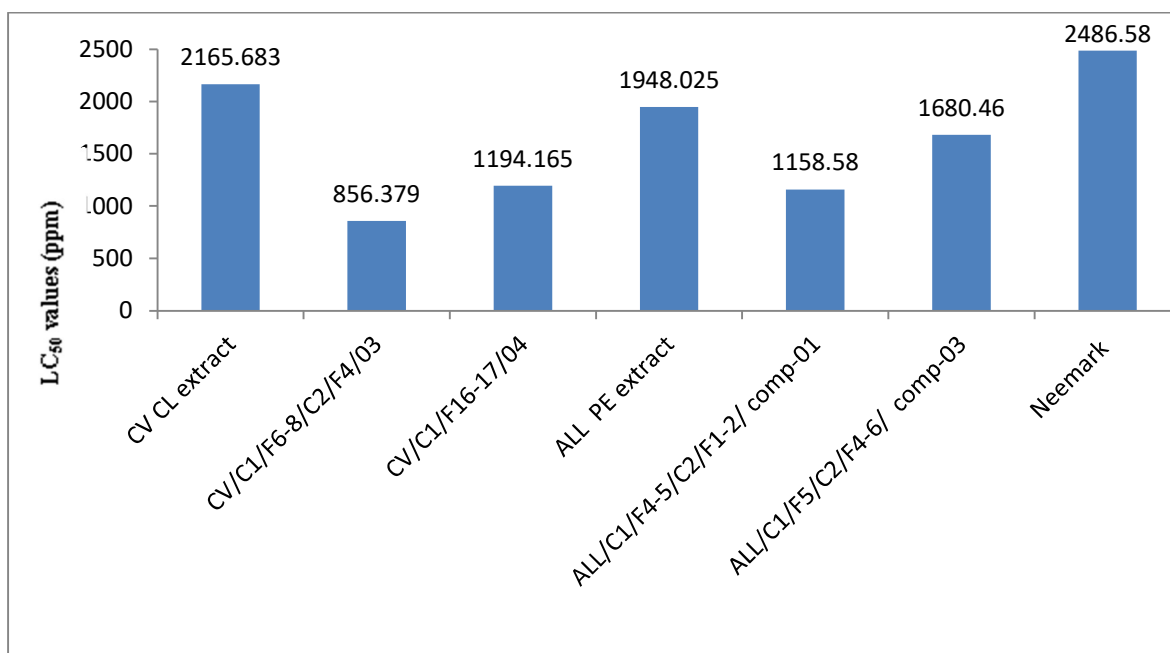


Figure No. 18a: Comparison of LC₅₀ values of CHE of *Cleome viscosa*, PEE of *Artocarpus lakoocha* Leaf and its isolated compounds on *Poecilia reticulata*

Comparatively, the LC₅₀ values observed on *Poecilia reticulata* for CHE of CVA was much higher than PEE of ALL. Hence the margin of safety for the use of CHE was significantly better than PEE of ALL. However, all the isolated compounds had similar LC₅₀ values in the range of 800 to 1000 ppm. Thus, it could be inferred that the CHE of CVA and all the isolated compounds are safer to be used concomitantly with *Poecilia reticulata*. The higher LC₅₀ values indicated a nontoxic nature of extracts and isolated compounds on non-targets.

4.8.1 Suitability Index (SI)/ Predator safety factor (PSF)

Suitability index (SI) or Predator safety factor (PSF) was calculated for each species of predator. The greater the SI/PSF, the greater the suitability of the extract/ isolated compound for integrated vector control. The results of SI/PSF are as in Table No. 22b and Figure No. 18b.

Table No. 22b: Suitability index (SI) of *Poecilia reticulata*

Extract/ isolated compound	SI/ PSF		
	<i>Anopheles stephensi</i>	<i>Culex quinquefasciatus</i>	<i>Aedes aegypti</i>
CVA CHE	50.47	200.83	18.55
CVA/C1/F6-8/C2/F4/03	112.83	116.67	64.83
CVA/C1/F16-17/04	44.21	20.13	32.03
ALL PE extract	8.07	3.12	1.68
ALL/C1/F4-5/C2/F1-2/comp-01	12.02	12.89	13.21
ALL/C1/F5/C2/F4-6/comp-03	5.41	14.71	15.09
Neemark (1% azadirachtin)	130.43	115.49	94.26
SI- Suitability index, PSF-Predator safety factor, CVA- <i>Cleome viscosa</i> Linn., ALL- <i>Artocarpus lakoocha</i> Roxb., leaves; CHE- chloroform, PE- Petroleum ether, C1- column 1; C2- column 2; F- Fractions, comp- isolated compound			

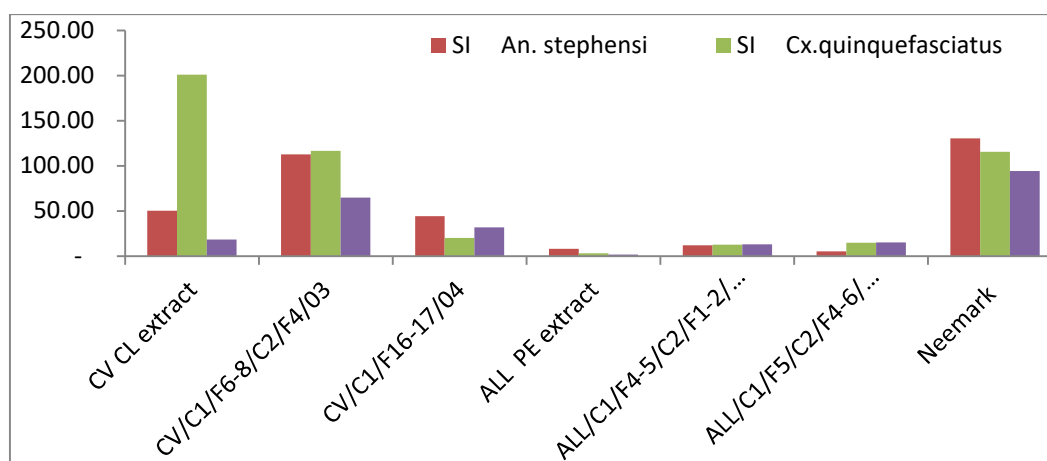


Figure No. 18b: Comparison of the Suitability index (SI- Suitability index, CL/CHE- chloroform, PE/PEE- Petroleum ether, C1- column 1; C2- column 2; F- Fractions, comp- isolated compounds, Neemark- standard 5% azadirachtin

CHE of CVA and its isolated compounds CVA/C1/F6-8/C2/F4/comp-03, CVA/C1/F16-17/comp-04 had higher SI values of 50.47, 112.83, 44.21 compared to PEE of ALL and its isolated compounds ALL/C1/F4-5/C2/F1-2/comp-01, ALL/C1/F5/C2/F4-6/comp-03 with 8.07, 12.02, 5.41 respectively if concomitantly treated with *An stephensi*.

Similar results could be compared for *Culex quinquefasciatus* and *Aedes aegypti* also. It was observed that compound **CVA/C1/F6-8/C2/F4/03** was as safe as Neemark (1% azadirachtin) on all three species. SI values of CHE of CVA was 90% better than Neemark (1% azadirachtin) on *Culex quinquefasciatus* which indicated greater susceptibility followed by *Anopheles stephensi* and *Aedes aegypti*.

4.9: Pesticidal Formulations from CHE of *Cleome viscosa*

The CHE was formulated as floating tablets, liquid formulation emulsions, and wettable powders as they are advantageous in several ways.

4.9.1: Floating tablets

Mosquito larvae float near the water surface for respiration. With floating tablets, respiration can be inhibited with synergistic action of larvicidal activity thus increasing the larval mortality. Formulation F1-4 (400 mg each) were punched in a tablet press following the wet granulation method using natural binders/ adjuvants like sodium alginate, xanthum gum, karaya gum, acacia, tamarind seed kernel gum. Citric acid and sodium bicarbonate to induce the floating properties. Fifteen formulations were formulated in preliminary trials. The FT-IR was taken to analyze the chemical interactions between extract and polymers and displayed no major shifts in the peaks indicating the non-reactive nature of the polymers and binding agent with CHE. The formulations, with no chemical interactions by IR spectra, were finalized and designated as F1-4 (Figure No. 19a and Table No. 23a).

The tablets were round, green brown in color, with an average thickness of 6mm and a diameter of 12mm. The weight variations and friability were within pharmacopeial limits of $\leq 5\%$ (Table No. 23b).

Table No. 23a: FT-IR of Chloroform extract of *Cleome viscose* and Formulation blend F1-4

Functional Groups	Frequency cm ⁻¹	
	CHE	F1-4
C-C	506.44	504.65
Ar-H	645.85	645.85
C—N	1294.77	1294.77
N=N	1348.56	1348.56
C=O	2344.42, 2482.55, 2656.99	2344.42, 2482.55, 2656.99
Ar-H	2977.64	2977.64
Ar-H	3362.34, 3486.96, 3596.58, 3600.32, 3806.43	3362.34, 3486.96, 3596.58 3600.32, 3806.43

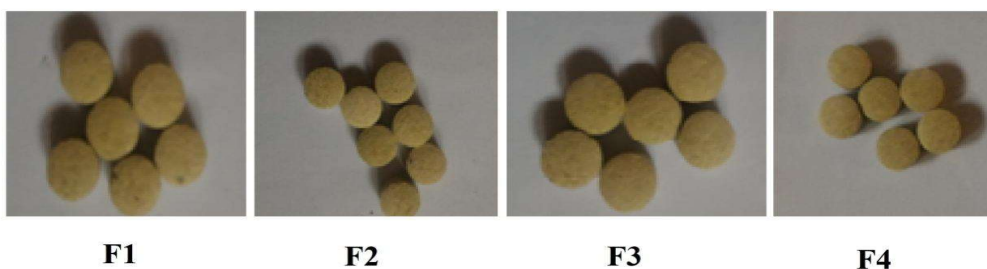


Figure No. 19a: Floating tablet formulations F1-F4 from CHE of *Cleome viscose*

***In vitro* buoyancy studies:** The least FLT and highest TFT were recorded with formulation F4. All three formulations had good buoyancy with a maximum floating lag time of 62- 116 seconds and a total floating time of 28- 38 hrs. (Table No. 23b and Figure No.19a, 19b).

Swelling Index (SI): The formulation F4 had the highest SI, and the swelling was directly proportional to the amount of HPMC K4M, sodium alginate, and xanthum gum and inversely to karaya gum. The imbibition and subsequent diffusion of the extract were due to an increase in the percentage of swelling (Table No. 23b).

Table No. 23b: Physico-chemical properties of floating tablets Chloroform extract of *Cleome viscosa*

Formulation	Shape	Color	Diameter (mm)	Thickness (mm)	Weight variation (mg)	Friability (%)	Drug Content (mg)	Hardness (kg/cm ²)	Swelling index				Floating/ Buoyancy	
									1	2	3	4	FLT(s)	TFT (hr)
F1	Round	Green - brown	12.15 ±0.014	6.12 ±0.012	405 ±0.24	0.71	199±0.34	6.2 ±0.57	20	26	48	58	116.0± 5	28 ± 2
F2			12.14 ± 0.021	6.01 ±0.010	396 ±0.56	0.66	198 ±0.23	5.6 ±0.84	25	39	42	48	84.25± 5	32 ± 2
F3			12.23 ±0.012	6.12 ±0.011	403 ±0.45	0.62	199± 0.68	4.8 ±0.70	33	38	43	49	62.75± 5	35 ± 2
F4			12.17 ±0.013	6.06 ± 0.020	404 ±0.76	0.62	200± 0.42	5.3±0.12	40	51	58	64	87.50± 5	38 ± 2

Figure no 19b: *In vitro* floating of tablet formulation-F4 at 0 minutes, 2 minutes and 24 hours

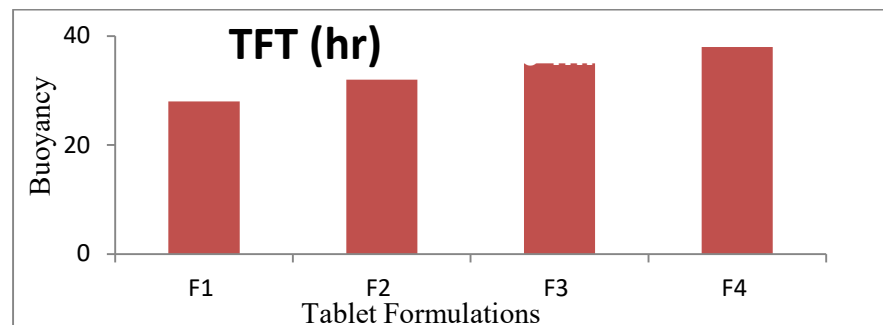


Figure No. 19c: Buoyancy- floating time of tablet formulations F1-4

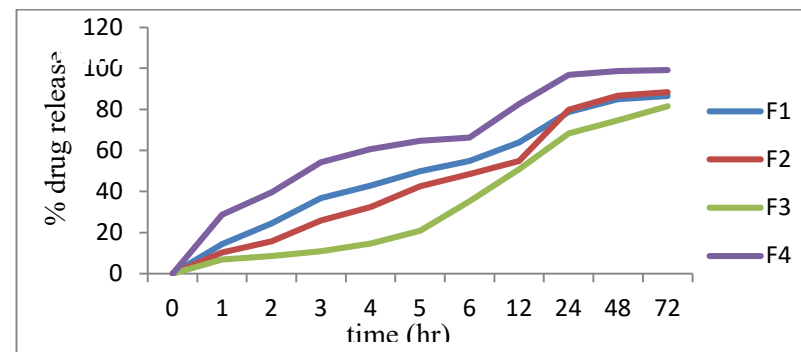


Figure No. 19d: Drug release profile tablet formulations F1-4 with time

4.9.2: Liquid concentrates

Emulsions are biphasic liquids for immiscible ingredients in water. The emulsions/ liquid concentrates were prepared to get an ideal o/w emulsion with an HLB value of 8-16. Surfactant tween 80, sorbitol, glycerine, tamarind seed gum, and gum acacia were incorporated to maintain the HLB value. Natural, inexpensive sunflower oil served as an oil phase to incorporate the CHE, and gallic acid and sodium benzoate acted as natural antioxidants.



Figure No. 20a: Emulsifiable concentrate formulations F5- F7 of CHE of *Cleome viscosa*

To improve the stability, an average globule size of $4 \pm 0.56 \mu\text{m}$, viscosity, and specific gravity were maintained. The pH was in the alkaline range to match the pH conditions of the mosquito breeding habitats. Physicochemical parameters are represented in Table No. 24. The prepared emulsion formulations were transferred into transparent glass vials and stored at RT for phase separation and the larvicidal activity was performed.

Table No. 24: Physico-chemical properties of emulsifiable concentrate formulations F5- F7 of Chloroform extract of *Cleome viscosa*

Formulation	Color	Odor	Appearance	particle size μm	Viscosity m^2/s	Specific gravity kg/m^3	pH	% drug content @ 24hr
F5	Dark	Chara	Colloidal	4	0.73	1.16	8.4	99.88
F6	green	cterist		4	0.83	1.13	8.2	98.56
F7		ic		5	0.73	1.10	8.8	98.28

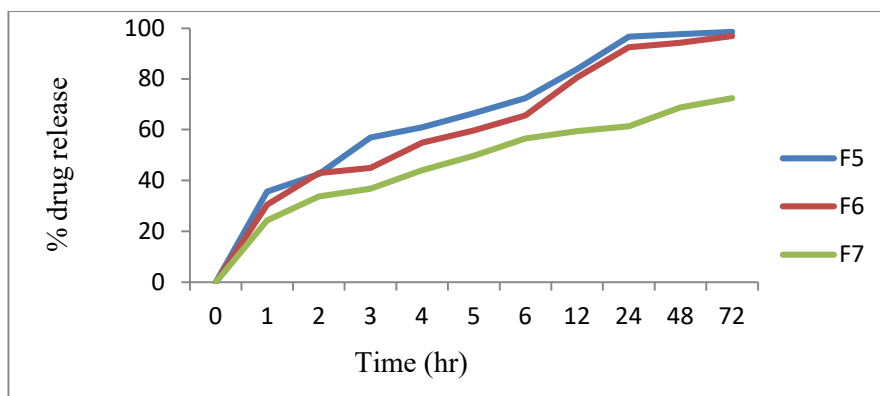


Figure No. 20b: Drug release profile of liquid concentrates F5-F7 with time

4.9.3: Wettable powders

Wettable powders F8- F10 were produced were tested for powder particle size and flow characteristics like bulk density tapped density, Hausner's ratio, Carr's index, and angle of repose. The spread ability and dissolution are influenced by ideal particle size and good flow properties. Moisture content was $\leq 3\%$, bulk density, tapped density Hausner's ratio, Carr's index, and angle of repose were within the pharmacopeial limits of an ideal powder (Table No. 25 and Figure No. 21a- 21c). The pH of 1% solution in water was neutral to alkaline range simulating larval habitats. Further, the stable and physico-chemically characterized formulations were subjected to larvicidal bioassay.



Figure No. 21a: Wettable Powder Formulation F10

Table No. 25: Physico-chemical properties of formulations F8- F10 of Chloroform extract of *Cleome viscose*

Wettable Powder Formulation	Moisture content % w/w	Bulk density (g/cm ³)	Tapped density (g/cm ³)	Hausner's ratio (%)	Carr's index (%)	Angle of repose	pH 1% solution	% drug content at 24 hrs
F8	0.26	0.28	0.33	1.16	13.86	24° 30'	6.8	99.26
F9	0.19	0.27	0.31	1.14	12.26	26° 25'	6.9	99.39
F10	0.10	0.28	0.32	1.13	11.64	24° 38'	7.1	99.65
F11	0.08	0.28	0.33	1.17	14.19	24° 84'	7.5	99.54
F12	0.05	0.28	0.34	1.18	15.43	29° 45'	7.8	99.47

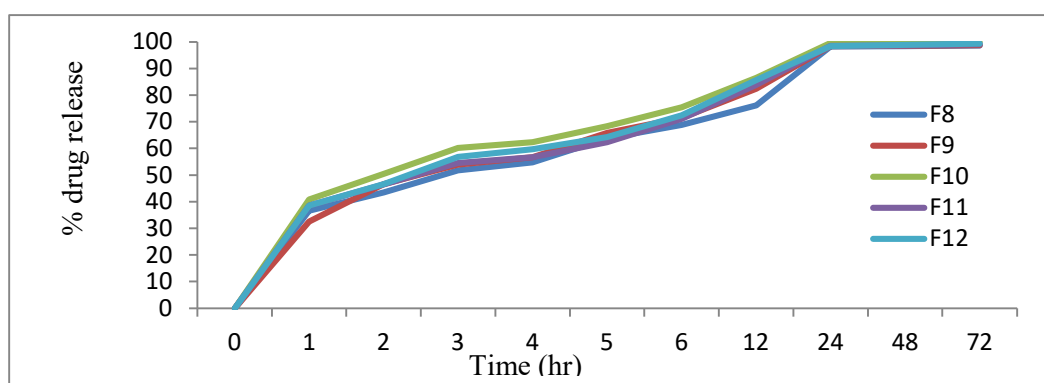


Figure No. 21b: Drug release profile of Wettable Powders F8-12 with ideal dissolution and extract release profile at different time intervals

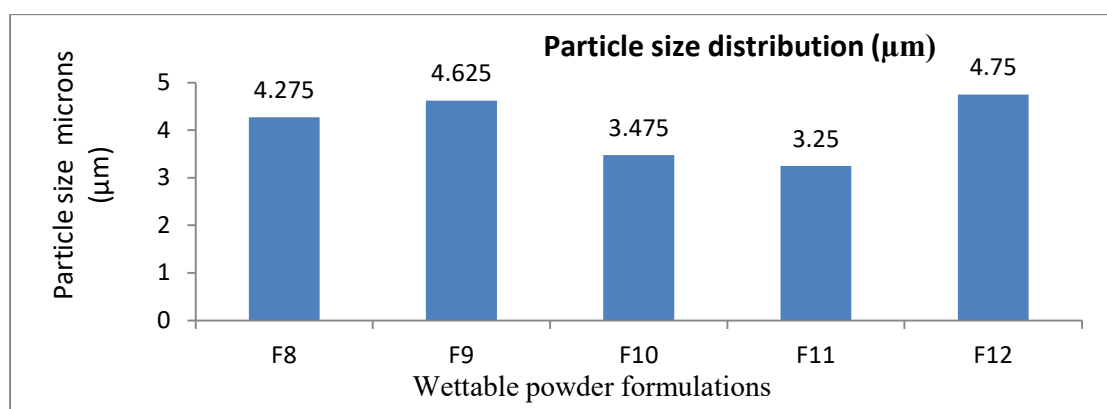


Figure No. 21c: Average Particle size of Wettable Powders F8-12, F10 with ideal particle size for flow properties

4.10: Larvicidal activity of Pesticidal Formulation F1-F12

The larvicidal activities of floating tablet formulations were screened for larvicidal activity against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. The results of the larvicidal activity are as in Table No. 26 and Figure No. 22. The lowest LC₅₀ values of 157.431, 40.151, 260.895 ppm were recorded for Formulation F4 compared to other tablet formulations and to that of the CHE of CVA, with LC₅₀ values of 141.99, 35.68, 386.32 ppm on *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* respectively. The larvicidal activity was proportional to the release of CHE from the tablet formulations.

Emulsifiable concentrates formulations F5- F7 of CHE of CVA were tested and the formulation F5 displayed least LC₅₀ values of 106.59, 46.23, 382.85 ppm compared to CHE of CVA and other formulations F6 and F7 on the three mosquito species respectively.

The powder formulations F8-12 screened for larvicidal activity against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* displayed least LC₅₀ values of 65.265, 17.995, 100.15 ppm for formulation F10 compared to other formulations F8-12 and CHE of *Cleome viscosa* on *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* respectively (Table No. 26). Figure No. 22 compares the overall larvicidal activity of formulations F1-F12.

Among tablet formulations, F4 had the highest larval mortality. Similarly, liquid formulations F5 had significant larvicidal activity. Formulation F10 of wettable powders was the most active among powders. Overall, formulation F10 wettable powder with a greater drug release profile had the highest larvicidal activity comparable to extract CHE of *Cleome viscosa*. Formulations F4, F5 and F10 were able to dissolve uniformly and produce larvicidal effects similar to extract CHE of *Cleome viscosa*.

Table No. 26: Larvicidal activity of formulations F1-F12 from Chloroform extract of *Cleome viscosa*

Formulation	Mosquito species	LC ₅₀	LC ₉₀	χ^2	Limits for LC ₅₀	
					LCL	UCL
F1	<i>Anopheles stephensi</i>	285.74	96.44	12.77	266.83	296.76
	<i>Culex quinquefasciatus</i>	198.84	68.92	34.65	174.63	245.83
	<i>Aedes aegypti</i>	385.32	63.85	15.53	354.26	394.42
F2	<i>Anopheles stephensi</i>	496.45	88.90	8.99	385.44	408.58
	<i>Culex quinquefasciatus</i>	356.85	74.66	10.56	324.56	384.55
	<i>Aedes aegypti</i>	543.71	56.97	7.83	396.34	560.62
F3	<i>Anopheles stephensi</i>	640.32	78.91	8.70	420.34	497.76
	<i>Culex quinquefasciatus</i>	416.58	12.33	12.73	384.56	450.66
	<i>Aedes aegypti</i>	742.56	44.75	10.79	694.56	760.45
F4	<i>Anopheles stephensi</i>	157.43	58.36	6.77	96.78	185.64
	<i>Culex quinquefasciatus</i>	40.15	94.56	9.75	26.33	60.63
	<i>Aedes aegypti</i>	260.89	45.62	12.62	152.67	284.65
F5	<i>Anopheles stephensi</i>	106.59	620.45	6.43	136.65	156.90
	<i>Culex quinquefasciatus</i>	46.22	350.58	2.88	40.78	338.90
	<i>Aedes aegypti</i>	382.85	867.45	6.91	360.58	459.67
F6	<i>Anopheles stephensi</i>	289.68	674.60	16.53	250.35	466.56
	<i>Culex quinquefasciatus</i>	96.45	406.66	12.74	88.12	134.78
	<i>Aedes aegypti</i>	596.74	856.53	10.45	532.45	627.44

F7	<i>Anopheles stephensi</i>	475.76	630.44	3.56	430.89	450.36
	<i>Culex quinquefasciatus</i>	389.74	496.45	6.23	360.78	398.63
	<i>Aedes aegypti</i>	694.86	898.06	4.67	638.36	732.43
F8	<i>Anopheles stephensi</i>	86.89	142.34	6.91	63.45	94.47
	<i>Culex quinquefasciatus</i>	45.89	134.67	3.56	41.33	49.21
	<i>Aedes aegypti</i>	178.89	220.23	15.53	172.76	186.56
F9	<i>Anopheles stephensi</i>	79.85	152.65	10.79	75.66	89.490
	<i>Culex quinquefasciatus</i>	38.47	134.90	12.62	34.37	56.45
	<i>Aedes aegypti</i>	153.76	222.30	7.83	148.58	164.30
F10	<i>Anopheles stephensi</i>	65.26	139.59	10.56	60.86	78.66
	<i>Culex quinquefasciatus</i>	17.99	144.95	6.23	15.10	12.64
	<i>Aedes aegypti</i>	100.15	226.77	2.88	96.34	106.46
F11	<i>Anopheles stephensi</i>	68.54	142.66	9.75	62.77	74.42
	<i>Culex quinquefasciatus</i>	20.47	134.45	3.56	18.96	29.68
	<i>Aedes aegypti</i>	114.87	196.56	2.88	108.34	134.55
F12	<i>Anopheles stephensi</i>	75.25	149.32	6.77	70.96	86.39
	<i>Culex quinquefasciatus</i>	25.38	138.17	12.74	24.63	32.49
	<i>Aedes aegypti</i>	128.47	238.90	6.43	116.58	143.45
F1-4- formulation 1-4, LC ₅₀ - Lethal concentration at which 50% mosquito larvae are dead, LC ₉₀ - Lethal concentration at which 90% mosquito larvae are dead, χ^2 - Chi-square values, LCL- Lower confidence limit, LCL-Upper confidence limit, (p ≤0.05)						

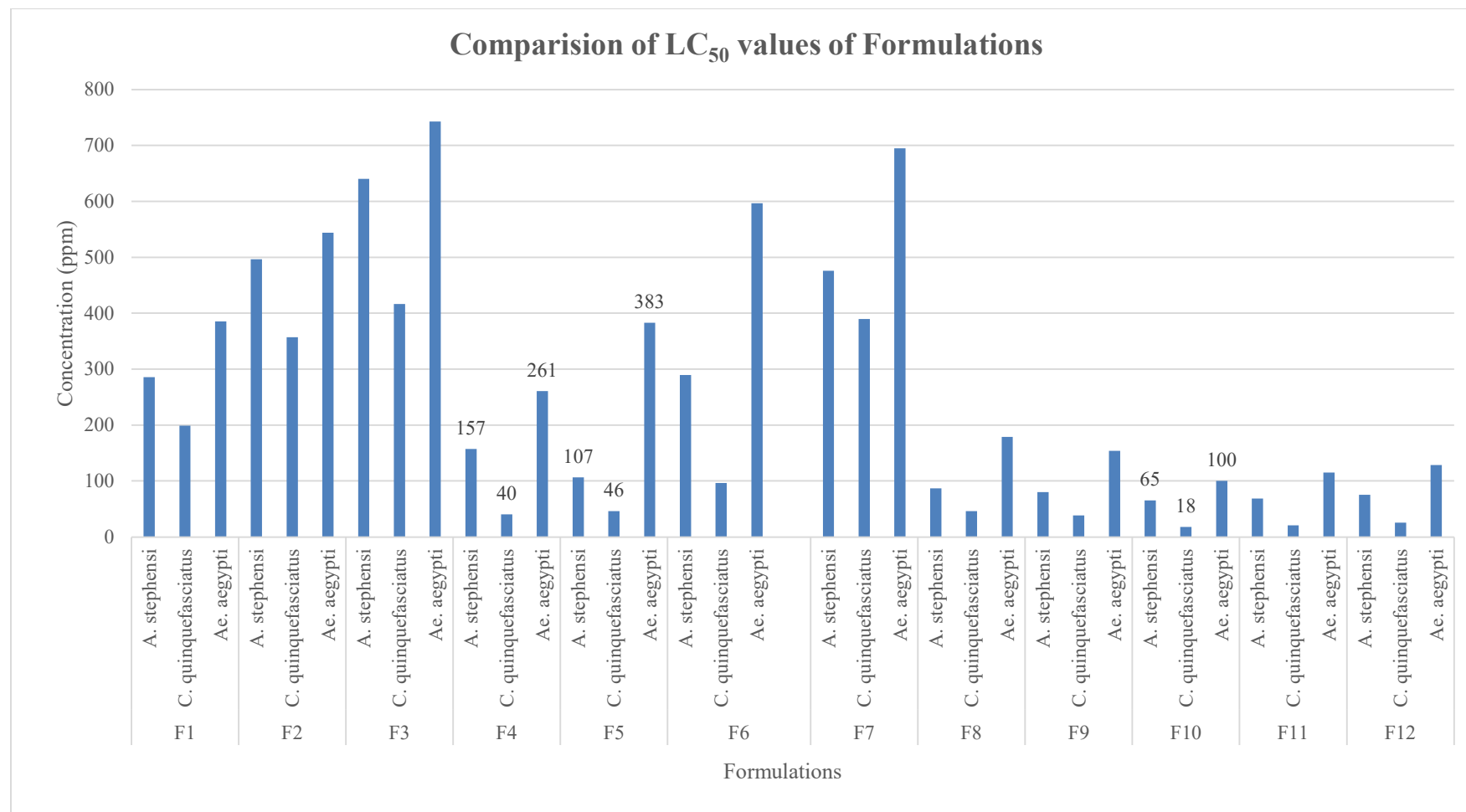


Figure No. 22: Comparison of LC₅₀ values of Larvicidal formulations F1-12

4.11: Stability Studies of Formulations

Formulations F4, F5, and F10 which showed the lowest LC₅₀ values with good drug release profile were subjected to stability studies at 28°C and 40°C at RH of 70% for 180 days (6 months) Table No. 27a, 27b, 27c. The larvicidal activity, drug release characteristics, and physicochemical parameters were established, at different time intervals on 1, 7, 14, 30, 60, 120, and 180 days. No significant changes in the physicochemical properties were observed in tablet formulation F4 and water-dispersible powder formulation F10. Liquid concentrate began to degrade with mild phase separation and emulsion cracking occurred at 40°C with significant changes in density and viscosity. Also, the LC₅₀ values were higher than tablet F4 and wettable powder F10. Table No. 28 and Figure No. 23 compares the LC₅₀ values of stability-tested formulations F4, F5, and F10 on *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* respectively at 28°C and 40°C. Formulation F4 and F10 with significant larvicidal activity were more stable even with changes in temperature and humidity.

Table No. 27a: Physicochemical evaluation: Stability studies Formulation F4

Condi on	Time	Sh ape	Color	Diameter (mm)	Thickness (mm)	Weight variation (mg)	Friabili ty (%)	Hardness (kg/cm ²)	Swelling index				Buoyancy evaluation	
									1	2	3	4	FLT(s)	TFT (hr)
Stabi lity studi es at 28 °C	Day 1	R	Gre	12.17 ±0.01	6.05 ±0.02	405± 1.85	0.64	5.3 ±0.11	41	52	56	65	87.5	38
	Day 7	o	en	12.17 ±0.01	6.04 ±0.02	406±2.82	0.63	5.3 ±0.12	42	51	54	66	86.75	40
	Day 14	u	bro	12.17 ±0.01	6.06 ±0.04	404±1.24	0.63	5.3 ±0.12	41	50	54	65	88.25	38
	Day 30	n	wn	12.17 ±0.01	6.04 ±0.04	405 ±2.49	0.63	5.3 ±0.12	42	52	56	64	88	36
	Day 60	d		12.16 ±0.09	6.05± 0.03	405 ±2.44	0.64	5.3 ±0.12	41	50	54	65	87	38
	Day 90			12.17 ±0.01	6.05± 0.03	405 ±2.45	0.63	5.3 ±0.13	40	54	55	64	88	38
	Day 120			12.17 ±0.01	6.05± 0.02	404 ±2.64	0.64	5.3 ±0.14	42	54	56	63	88	39
	Day180			12.17 ±0.01	6.04± 0.03	405 ±2.38	0.63	5.3 ±0.13	46	56	58	68	87	38
Stabi lity studi es at 40 °C	Day 1	R	Gre	12.17 ±0.01	6.05± 0.03	403±1.9	0.60	4.9 ±0.69	40	48	54	63	88	34
	Day 7	o	en	12.17 ±0.01	6.05 ±0.03	403±2.95	0.58	5.4 ±0.12	41	52	56	62	87.75	36
	Day 14	u	bro	12.17 ±0.01	6.06 ±0.03	403±1.57	0.58	5.3 ±0.12	42	52	58	62	88	38
	Day 30	n	wn	12.17 ±0.01	6.06 ±0.04	402±0.70	0.59	5.0 ±0.13	43	46	56	64	88	36
	Day 60	d		12.17 ±0.01	6.05± 0.02	405 ±2.49	0.64	5.3 ±0.12	43	50	54	66	88	38
	Day 90			12.17 ±0.01	6.04± 0.03	403 ±2.62	0.62	5.2 ±0.13	41	50	54	65	87	37
	Day 120			12.17 ±0.01	6.05± 0.02	403 ±2.46	0.63	5.3 ±0.12	44	52	58	66	89	38
	Day180			12.17 ±0.01	6.05± 0.03	403 ±2.22	0.66	5.4 ±0.15	43	54	58	68	89	38
*All values are expressed as mean with SD of <5% (p≤0.05)														

Table No. 27b: Physicochemical evaluation: Stability studies of Formulation F5

Temperat ure	Days	Color	Odor	Appea rance	Viscosity v/v	Specific gravity g/m	pH	Microbial growth	% drug content
28℃	Day 1	Dark green	Charac teristic	Collo idal	0.73	1.05	8.4	Nil	94.21
	Day 7				0.76	1.01	8.4		93.97
	Day 14				0.86	1.01	8.6		89.45
	Day 30				0.82	1.00	8.6		84.36
	Day 60				0.79	1.00	8.6		80.24
	Day 90				0.79	1.09	87		80.89
	Day 120				0.77	1.06	8.6		80.89
	Day180				0.76	1.04	8.7		80.96
40℃	Day 1	Dark green	Charac teristic	Collo idal	0.86	1.00	8.4	Nil	92.21
	Day 7				0.82	1.00	8.3		90.27
	Day 14				0.79	1.03	8.2		86.46
	Day 30				0.76	1.01	8.2		82.64
	Day 60				0.79	1.00	8.2		88.69
	Day 90				0.76	1.04	8.2		88.22
	Day 120				0.76	1.05	8.2		88.62
	Day180				0.79	1.06	8.2		87.11
*All values are expressed as mean with SD of <5% (p≤0.05)									

Table No. 27c: Physicochemical evaluation: Stability studies of wettable powder formulation F10

Temperat ure	Days	Colo r	Odor	Moisture content % w/w	Bulk density (g/cm ³)	Tapped density (g/cm ³)	Hausner' s ratio (%)	Carr's index (%)	Angle of repose	pH 1% sol	% drug content at 24hrs
28 °C	Day 1	Gr een	Char acteri stic	0.10	0.28	0.31	1.13	11.63	24° 38'	7.1	99.62
	Day 7			0.11	0.27	0.32	1.20	16.69	25° 25'	7.2	99.56
	Day 14			0.12	0.28	0.33	1.16	14.24	24° 28'	7.2	99.58
	Day 30			0.12	0.28	0.32	1.13	12.03	24° 66'	7.4	99.88
	Day 60			0.11	0.28	0.32	1.16	14.15	25° 42'	7.4	99.64
	Day 90			0.12	0.28	0.32	1.16	14.16	25° 42'	7.6	99.94
	Day 120			0.12	0.28	0.32	1.16	14.26	25° 62'	7.7	99.69
	Day180			0.12	0.28	0.32	1.16	14.16	24° 65'	7.8	99.84
40 °C	Day 1	Gr een	Char acteri stic	0.10	0.27	0.3	1.18	15.31	24° 39'	7.1	99.85
	Day 7			0.09	0.27	0.34	1.18	15.43	25° 35'	6.9	99.84
	Day 14			0.11	0.28	0.33	1.16	14.50	24° 98'	7.2	99.42
	Day 30			0.09	0.27	0.32	1.17	14.59	24° 96'	7.3	99.65
	Day 60			0.09	0.24	0.32	1.18	15.38	25° 35'	7.4	99.75
	Day 90			0.08	0.27	0.32	1.18	15.38	25° 36'	7.3	99.74
	Day 120			0.09	0.27	0.33	1.17	15.39	25° 44'	7.2	99.78
	Day180			0.09	0.27	0.33	1.18	15.39	25° 66'	7.8	99.75
*All values are expressed as mean with SD of <5% (p≤0.05)											

Table No. 28: Larvicidal Activity of Formulations F4, F5, F10 during Stability Studies

Day	Sample/ Formulation	<i>Anopheles stephensi</i>		<i>Culex quinquefasciatus</i>		<i>Aedes aegypti</i>	
		LC ₅₀ values*		LC ₅₀ values*		LC ₅₀ values*	
		@28°C	@40°C	@28°C	@40°C	@28°C	@40°C
Day1	<i>Cleome viscosa</i>	141.99	140.25	35.68	35.54	386.32	385.86
Day 7		139.94	140.28	35.47	35.58	384.78	384.45
Day 14		140.25	140.25	36.56	36.29	378.58	376.89
Day 30		138.25	140.58	37.25	36.92	384.24	384.88
Day 60		138.14	140.20	36.13	36.96	386.44	388.67
Day 120		142.22	144.45	37.96	36.67	388.67	388.94
Day1	Floating Tablet F4	157.43	169.81	40.15	65.74	260.89	431.09
Day 7		155.83	183.65	48.42	63.45	448.16	522.61
Day 14		157.44	181.82	48.42	63.51	448.19	470.64
Day 30		157.48	187.43	49.09	68.37	448.22	481.31
Day 60		157.56	187.88	49.78	68.69	448.78	481.18
Day 120		157.45	187.23	49.64	68.82	448.55	481.22
Day1	Emulsifiable concentrate F5	106.59	167.28	46.23	72.65	382.85	473.72
Day 7		121.09	181.62	71.30	86.38	472.96	491.85
Day 14		123.63	199.42	72.02	88.58	599.79	495.43
Day 30		121.02	248.01	92.42	95.39	575.81	612.89
Day 60		140.77	248.16	92.34	95.75	575.56	612.28
Day 120		141.34	248.67	92.56	95.66	575.98	612.67
Day1	Wettable powder F10	65.26	108.79	17.99	26.51	100.15	236.73
Day 7		105.63	111.14	19.57	17.16	253.46	228.74
Day 14		105.79	109.22	23.62	22.21	265.15	240.35
Day 30		105.56	106.12	21.63	26.31	263.96	240.58
Day 60		105.77	106.17	21.66	26.35	263.78	240.34
Day 120		105.28	106.55	21.59	26.38	263.75	240.00

*All values are expressed as mean with SD of <5% (p≤0.05)

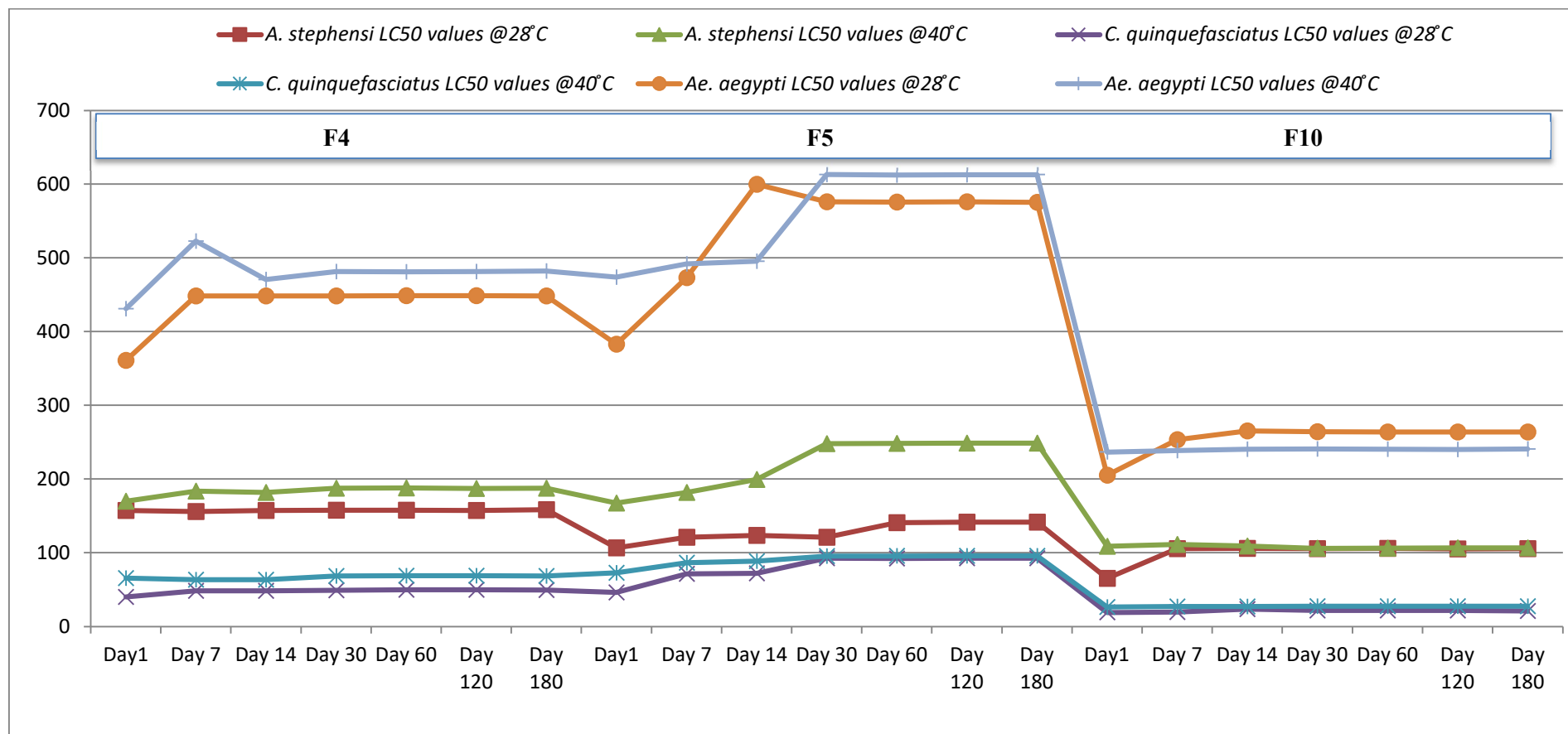


Figure No. 23: Larvicidal activity of formulations F4, F5, and F10 maintained at 28°C and 40°C on *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*

DISCUSSION

Mosquitoes are known as tiny assassins because they spread viral and parasite illnesses, which cause a high rate of human mortality worldwide¹⁻². Over 2.5 million deaths and deformities due to vector-borne diseases are reported by the World Health Organization each year³. Many countries strategically approach vector control to prevent infectious disease dynamics and unexpected outbreaks⁴. Such approaches often involve using synthetic pesticides. However, its prolonged and careless has increased resistance and adverse effects on organisms other than the intended targets⁵⁻⁶.

Man has utilized medicinal plants for their pharmacological properties as cures for eons. Plants switch to the mevalonate or shikimic acid pathway from their regular metabolism to make secondary metabolites⁷⁻⁸. Plants release natural substances known as pesticides to ward against infestations by insects and grazing animals. The benefits of plant-derived pesticides over synthetics are astounding. Characteristics like biodegradability, non-development of resistance, synergistic effects, potentiation, absence of impacts on non-target entities, and preservation of ecological equilibrium. Traditionally and in folklore, plants including tobacco, pyrethrum, chrysanthemum, and neem are employed as insecticides⁸⁻⁹. The Industrial Revolution and industrialization led to the disdain of plants as insecticides and pesticides. Natural pesticides have few or no drawbacks are back in place. Therefore, our focus of vector control research has been the insecticidal screening of selected plants.

Authentication of plant materials is the first and foremost step in research related to plant drugs to confirm the identity and taxonomical status of the plant, and hence the plants were authenticated plant voucher specimens were deposited in the herbarium.

5.2: Standardization of plant materials

Plant-based medicines formed the basis of modern allopathic drugs. With time, traditional knowledge was underestimated, neglected, and quickly forgotten due to invasions, wars, lifestyles, and industrialization. As the new charms of allopathy are fading, people are reverting to conventional and folklore systems. Evidence-based strategies to prove pharmacology of herbal drugs should be taken up. Standardization is the first step to obtaining a comprehensive monopoly in the pharmacological treatment of herbal medications, products, and goods⁸⁶. Herbal drugs are concerned with the quality and validation of methods. Thus, standardization parameters are important aspects for establishing the medicinal uses and pharmacological activities of natural and plant drugs. The quality and purity of drugs vary dramatically and are affected by various factors such as time, harvest season, place, and geographical factors- climate, soil, rainfall, humidity, harvest method, processing, and storage. The nature of phytoconstituents is affected in different ways by the discussed factors. Hence, the World Health Organization (WHO) has set quality control parameters to evaluate drugs of natural origin. Monographs are developed over time to establish the drug profile. Natural drug and herbal products must adhere to standards of quality, purity, safety, and efficacy⁸⁷.

In this view, standardization of CVA, ALL, and ALF was initiated by evaluating the pharmacognostic and physico-chemical parameters. Standardization will set the first step to establishing quality and quantifying the active constituents. Morphological, microscopical, ash values, extractive values, foreign organic matter, moisture content, fluorescence analysis, etc. were calculated/ observed and compared with general indicators prescribed in WHO guidelines for quality control parameters.

Lower ash values viz., total ash, water soluble ash, and acid insoluble ash were within acceptable limits. This indicated that the samples were less or uncontaminated by adherents and adulterants such as carbonates, bicarbonates, phosphates, silica, and sand particles¹⁶⁶⁻¹⁷². Fluorescence analysis is a ready and valuable tool in the identification, authentication, and pharmacognostical evaluation of herbal drugs that can be applied as a quality control tool when analytical methods are limited or not available. The chemical interaction of phytoconstituents with common reagents can be observed under fluorescent light^{168, 172-174}. Based on these parameters, the plant materials were free from organic and inorganic contaminations.

The plant materials were selectively extracted with solvents, successively, in the increasing order of polarity and low toxicity profiles. From the extraction, yield and extractive values were noted. Extractive values gave a vague idea of the quality and quantity of phytoconstituents. The highest extraction yield in CVA was for the water followed by ethanol, chloroform, and petroleum ether. A similar pattern of phytochemical solubility with water was noticed with the leaves and fruits of AL in comparison to other solvents. Aprotic solvents like petroleum ether and chloroform extracted the nonpolar components like- terpenes, terpenoids, and steroids, and protic solvents like ethanol and water extracted polar constituents such as glycosides, tannins, phenolics, proteins, and carbohydrates.

5.2.5: Phytochemical analysis

The alkaloids in chloroform extract, steroids, triterpenoids, fixed oils in PEE, tannins, flavonoids, phenolics in ETE, and proteins and carbohydrates were identified in the WE of CVA. Similarly, alkaloidal glycosides, steroids, triterpenoids, tannins, flavonoids, and phenolics in the ethanolic extract, and the proteins, sugars, and carbohydrates were

identified as phytoconstituents in the aqueous extract of ALF as reported by many authors^{162, 175-177}.

5.3: Physical Properties and Percentage Yield

Physicochemical evaluation of extracts indicated the nature of the extract, color, consistency, and the percentage yield of extracts. These parameters could be used for the standardization of plant raw material as well as its extracts.

Further, the nature of the extract determines the proportion of fillers to be used to commercialize the extracts in the herbal extract industry¹⁷⁸. The percentage yield parameter is crucial for method validation and costing calculations of herbal extract. Solubility and pH of extract determine the type of drying process for commercial-scale processes¹⁷⁸⁻¹⁷⁹.

5.4: Collection and laboratory rearing of mosquito species

Disease-free mosquito larvae cultures can be and maintained for several generations in a laboratory. All the stages in the life cycle of mosquitoes are targets for mosquito control/ pesticidal activity. Late 3rd/ early 4th instar larvae which are sensitive to larvicides and biopesticides was chosen for larvicidal activity. Fully gravid mosquitoes were selected for oviposition deterrence activity. Late 2nd instar larvae were selected for juvenile hormone-mimicking insect growth regulatory activity.

Overall, the aim was to interrupt every possible stage in the life cycle of mosquitoes and achieve targeted mosquito pesticidal activity. The culturing and maintenance were done by following the standard protocol as per WHOPES. Larvae were maintained under controlled thermal regulation at an optimum temperature of $27 \pm 2^\circ\text{C}$, RH of $60 \pm 5\%$, and photoperiod of 14:10 (L: D) hrs.

5.5: Larvicidal Bioassay: Preliminary screening and toxicity studies for larvicidal activity

Aerial parts of *Cleome viscosa* Linn: In preliminary screening, the larvicidal activity of successive solvent extracts was tested for preliminary larvicidal bioassay. In comparison, the CHE had the highest larvicidal activity at the lowest concentrations tested for the three vector species *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes Aegypti*. A 100% effective concentration of 150 ppm for CHE was significant and higher than standard azadirachtin with similar morphological toxicities. To find the narrow lethal concentration range, a toxicity assay was performed. In a study reported by Sakthivadivel et al., 2014, CHE of *Cleome viscosa* had the maximum activity with LC₅₀ of 427.68, 455.92, 634.44 ppm at 24 hrs and 55.50, 24.25, 36.86 ppm at 48 hrs on *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* respectively¹⁸⁰. In another study by Islam et al, chloroform extracts of *Cleome viscosa* had larvicidal effects on adult beetles of *T. castaneum* at LD₅₀ concentrations as low as 0.170 mg/L¹⁸².

***Artocarpus lakoocha* Roxb.:** In leaves and fruits of AL, PEE had the highest and most significant dose-dependent larvicidal activity on all three mosquito species compared to other solvent extracts. *Ar. blancoi*, a related species of *Artocarpus* was reported for larvicidal activity LD₅₀ with ethyl acetate at 18.59 ppm, ethanol 411 ppm, hexane (685 ppm), and aqueous (20,158 ppm) fractions. Shailendra et al. reported the insecticidal activity of methanolic extract of fruit pericarp on the 2nd and 3rd instar larvae of *Aedes aegypti*. A mortality of 85% on the third day at a 100mg/ml dose and a lower dose (10 mg/ml) reported no mortality¹⁸³. Qualitative phytochemical screening of ethyl acetate extract revealed the presence of glycosides, sterols, triterpenes, flavonoids, saponins, and tannins. In our study, the PEE of ALL had sterols, triterpenes, flavonoids, and

triterpenoid saponins due to which a high mortality of 100% were observed at >200 ppm at 24 hrs.

Rajamma et al. reported the larvicidal of petroleum ether *Ocimum* species, where the bio-actives mainly composed of terpenes, terpenoids, steroids and triterpenoids with the LC₅₀ value of 39.31 and 40.02 on *O. basilicum* and *O. sanctum* oil respectively.

All the above-referred studies concluded that the low polarity of solvents such as petroleum ether, hexane, and chloroform played a crucial role in extracting phytoconstituents with larvicidal properties.

Toxicity study observations recorded in the study at various time intervals of 24, 48, and 72 hours help us to establish a diagnostic concentration to assess the susceptibility to larval species other than those tested here. This correlation shall help to prevent resistance in natural habitats under practical field conditions.

5.6: Oviposition Deterrence Activity

The oviposition in the life cycle of mosquitoes is a critical step in determining the population dynamics and endemics of vector-borne disease in a specific topographical location. The CHE of CVA has significant activity with low ER₅₀ values compared to other successive solvent extracts in all three mosquito species tested. The 100% deterrence was recorded at lower than 50 ppm for CHE of CVA followed by PEE. Thus, the CHE was the most effective Oviposition deterrent compared to other extracts tested. Radhika et al. and Warikoo et al reported the oviposition deterrence of the CHE extracts of *Ar. mexicana* extracts with similar observations with deterrence in the range of 50 - 100 ppm. The least egg deposit number of Zero and the percentage were recorded at 10 ppm. These results are in comparison with other pesticidal drugs such as DEET¹⁸⁸ and A13-37220¹⁸⁹ and plant extracts such as *Solanum trilobatum*¹⁹⁰, *Pongamia pinnata*¹⁹¹,

*Coleus forskohlii*¹⁹², and *Datura stramonium*¹⁹³ with similar reported phytoconstituents.

Out of all the extracts of ALL screened, only the PEE showed effective deterrence with low ER₅₀ values of less than 100 ppm on *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. PEE extracts of many plants PEE such as *Vitex negundi*¹⁸⁶, and *Tribulus terrestris* L¹⁸⁷ had 100% deterrence at 50-200 ppm and are significantly higher than PEE of ALL. Thus, PEE of ALL could be considered with better oviposition deterrent than other solvent extracts. It was also observed that, *Anopheles stephensi* and *Culex* were more susceptible to PEE of ALL than *Aedes aegypti*.

The PEE of ALF had significantly lower ER₅₀ values, and the 100% deterrence of PEE was recorded at 700, 1100, and 2000 ppm for three species respectively. The chloroform, ethanolic, and aqueous extracts displayed no or zero oviposition deterrence and hence had no effects on oviposition. In a study by Chinnamma et al PEE of *Vitex negundo* leaves were tested on insect species *Callosobruch chinensis* were susceptible to solvent extracts similar to that of PEE of ALL. The results indicated that non-polar solvents such as petroleum ether, chloroform, and acetone extract contain steroids, terpenes, triterpenoids, flavonoids and other phytoconstituents that deterred insects irrespective of insect taxonomical status.

These results are also conclusive that non-polar solvent, extract maximum phytoconstituents that determine the percentage of oviposition deterrence¹⁸⁵. Other observations such as the non-hatchability of the eggs due to microscopical denaturation and destruction of cell membranes, amniotic sac, etc.; and higher mortality of gravid females due to contact mosquitocidal effect and forced retention of eggs were significant in the CHE of CVA and PEE of ALL compared to other solvent extracts.

CHE and PEE also had mosquitocidal effects on the unhatched eggs and the adults similar to as reports of Reagan et al., and Prajapati et al., on *Aedes aegypti* L. and *Culex quinquefasciatus*¹⁹²⁻¹⁹⁴. In conclusion, the chloroform extract of CVA was the most effective as an oviposition deterrent followed by Petroleum ether extract of ALL among the other successive solvent extracts. The order of oviposition deterrence observed was CHE of CVA > PEE of ALL > PEE of ALF with sensitivity of *Anopheles stephensi* > *Culex quinquefasciatus* > *Aedes aegypti*.

5.7: Juvenile hormone-mimicking / insect growth regulation

Insect Growth Regulators (IGRs) are class of compounds that interfere with the growth, development, and metamorphosis of arthropods. The juvenile hormone (Neotinin) is a natural hormone secreted by *Corpora allata* in the insect brain. The JH analogs (JHA) from plant extracts mimic the natural growth process but keep the larvae in juvenile condition or induce super molting effects by skipping the intermediate stages of development to produce small undeveloped adults. The changes produce larval-pupal and pupal-adult intermediates, which result in the mortality¹⁰¹⁻¹⁰⁴.

The JH effects discussed earlier were profound and significant in some of the extracts of CVA, ALL, and ALF. The chloroform extracts of CVA had significant JHA activity compared to other solvent extracts of CVA. With AL, the PE extract of ALL had maximum activity with low EI₅₀ values compared to the PEE of ALF. In the control, larvae lasted approximately 6-8 days before emerging. The dose-dependent inhibition of emergence as larval mortality was prominent at 100- 400 ppm. Other observations included mortality as un-pigmented pupae was significant at 10, 25, and 50 ppm. PEE was more effective than CHE and ETE. In general, low polar solvent extracts, such as hexane, petroleum ether, chloroform, ethyl acetate etc., extract plant phytoconstituents such as terpenes steroids, triterpenoids, and low molecular weight phenolics and

flavonoids during extraction. These phytoconstituents have functional groups and structures that are similar to natural Juvenile Hormones I-IV found in insects. The hormonal imbalance ensures under or super-molting effects of insects allowing the instar larvae to stay immature and prevent converting into mature adults.

From the results, it's evident that chloroform of CVA and PEE extract ALL have larvicidal bioassay, juvenile hormone-mimicking activity, and oviposition deterrence activity. Studies on *Atlantia monophylla*, extracts by Sivagnaname et al. reported LC₅₀ values of 0.14 ppm and EI 50 of 0.05 ppm as insect growth regulators with larvicidal activity¹⁰². Martianasari et al. also reported that *Piper betel* L. essential oil could be used as a bio-insecticide to control *Aedes aegypti* in various ways as a larvicidal and IGR¹⁰³. The two studies mentioned above prove that plant extracts could have multi-modal pesticidal activity such as larvicide, oviposition deterrence IGR activity which are beneficial to controlling mosquito populations at different stages of the life cycle of mosquitoes. The selected plant extracts with multiple modes of insecticidal action can be formulated/ commercialized. Bioactivity-guided isolation of larvicidal compounds was also taken up to identify biomarkers in the selected extracts.

5.8: Bioactivity Guided Isolation of Larvicidal Compounds

The isolation of chemical compounds from plants guided by biological activities is called Bioactivity Guided Isolation. It a significant approach for developing new drug leads from natural products^{55- 56, 129}.

5.8.1: Bioactivity-guided Isolation of Larvicidal Compounds from Chloroform Extract of CVA

Bioactivity-guided isolation carried out on the chloroform extract of CVA resulted in 15 initial fractions. At 100 ppm, fraction **CVA/C1/F6-8** showed maximum larvicidal activity with % mortality of 82, 100, and 100% on *Anopheles stephensi*, 84, 100, 100%

mortality on *Culex quinquefasciatus* and 46, 88, 100% mortality on *Aedes aegypti* at 24, 48, and 72 hrs respectively. Fraction CVA/C1/F16-17 also displayed significant larvicidal activity with a % mortality of 60, 78, and 90 % on *Anopheles stephensi*, 48, 56, and 76 % mortality on *Culex quinquefasciatus*, and 28, 45, 62 % mortality on *Aedes aegypti* at 24, 48, and 72 hrs respectively. **Isolated compounds CVA/C1/F6-8/C2/F1/comp-01, CVA/C1/F6-8/C2/F2/comp-02, CVA/C1/F6-8/C2/F3/comp-03** from fraction **CVA/C1/F6-8** and CVA/C1/F16-17/comp-04 were subjected to larvicidal bioassay at concentration of 10- 50 ppm in triplicates. Mortality was recorded hourly for the first 6hrs, then for every 6hrs up to 72 hrs. CVA/C1/F6-8/C2/F1/comp-01 and CVA/C1/F6-8/C2/F2/comp-02 were inactive as larvicidal compounds.

CVA/C1/F6-8/C2/F3/comp-03 and **CVA/C1/F16-17/comp-04** had significant larvicidal activity at a concentration as low as 10ppm with 60, 20 at 6 hrs 80, 30% at 12 hrs and 100, 60% mortality at 24hrs. 100% mortality at 48 hrs was noted for both isolated compounds. *Anopheles stephensi* and *Culex quinquefasciatus* were more sensitive than *Aedes aegypti* to isolated compound CVA/C1/F6-8/C2/F4/03, while for compound CVA/C1/F16-17/04 a mixed sensitivity was observed. This kind of variation in sensitivity of the same plant and the same larvicidal compounds has been noted by several researchers¹⁹⁶⁻¹⁹⁷. From the literature survey of CVA, it was found that compounds, Cappariloside A and cappariloside B and lupeol isolated had larvicidal activity on *Aedes aegypti* at 24 hrs with LC₅₀ of 71.14, 99.79, 133.03 ppm. In contrast, an LC₅₀ of 158.71 ppm was noted at 48 hrs for Lupeol¹⁹⁵.

5.8.2: Bioactivity-guided Isolation of Larvicidal Compounds from Petroleum ether extract of ALL

In bioactivity-guided isolation of larvicidal compounds from the PEE of ALL, 13 initial fractions were pooled. Larvicidal activity at 100 ppm fraction ALL/C1/F5 had a

maximum activity of 64%, 92%, and 100% on *Anopheles stephensi*; 62%, 90%, 100% on *Culex quinquefasciatus* and 57, 92, and 98 % on *Aedes aegypti* at 24, 48, and 72 hrs respectively.

Active fraction ALL/C1/F5 was re-fractionated, and three compounds were isolated viz., ALL/C1/F5/C2/F1-2/comp-01, ALL/C1/F4-5/C2/F3/comp-02, and ALL/C1/F4-5/C2/F4-6/comp-03. The compounds ALL/C1/F5/C2/F1-2/comp-01 and ALL/C1/F5/C2/F4-6/comp-03 had a maximum larvicidal activity of 71.453, 66.639, 64.994 ppm, and 181.190, 183.279, 180.162 ppm on *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* at 24 hours. Similarly, Ajugalide-E, a phytoecdysteroid isolated by bioactivity-guided fractionation from the hexane fraction of *Aju chamaecristatus*. LC₅₀ of 95.66 ppm and 100% in the concentration of 102 ppm on *Anopheles stephensi* for the isolated compound¹⁹⁸. In a study by Deore et al., crude saponins, and purified saponins were tested on *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*. Further, some suggest the mechanism of toxicity may be due to the possible interaction of larvicidal compounds with the larval cuticle membrane and the disarranging of the membrane¹⁹⁹. Many authors have isolated certain classes of saponins from AL that might or might not represent larvicidal compounds.

5.9: Characterization: Structural elucidation of isolated compounds

Compound CVA/C1/F6-8/C2/F4/comp-01 was a flavonoid glycoside, isorhamnetin 3-*O*- β -d-glucopyranoside²⁰⁰. Compound CVA/C1/F6-8/C2/F4/comp-02 was determined as Quercetin 3-*O*-(2'-acetyl)-glycoside²⁰¹. Compound CVA/C1/F6-8/C2/F4/comp-03 was 1- S- [N-(sulonatoxy) but-3-nimidoyl]-1-thio-beta-D glucopyranose or Sinigrin (allyl isothiocyanate)²⁰². CVA/C1/F16-17/04 was 5- hydroxy 1, 4 naphthoquinone di-one- 6- beta – D glucopyranose e or Juglone-3-D-glucopyranose²⁰³. ALL/C1/F4-5/C2/F1-2/comp-01 was identified as steroidal compound Cycloartenol²⁰⁴ ALL/C1/F4-

5/C2/F3/comp-02 was identified as 2-arylbenzofuran derivative Lakoochin A²⁰⁵ and ALL/C1/F4-5/C2/F1-2/comp-03 as albafrican B²⁰⁶.

5.10: Nontarget organism: Guppy Fishes

Poecilia reticulata are exotic fish that prey on *Culex*, *Aedes*, and *Anopheles* larvae. It's widely used in the biological control of mosquito larvae. A major criterion for a larvicidal agent is that it should be safe and non-toxic to non-target organisms. So that ecological balance is maintained in the application sites.

The toxicity of CHE of CVA, PEE of ALL, and isolated compounds was tested on *Poecilia reticulata*. LC₅₀ values of 7165.68, 856.37, and 1194.16 ppm on *Poecilia reticulata* were noted for CHE of CVA, CVA/C1/F6-8/C2/F4/03, and CV/C1/F16-17/04, respectively. For PEE of ALL, ALL/C1/F4-5/C2/F1-2/comp-01, and ALL/C1/F5/C2/F4-6/comp-03 the LC₅₀ values of 1948.02, 858.58, and 980.46 ppm were observed. LC₅₀ values for fishes were much higher than the LC₅₀ values of target mosquito species. It can be inferred that the extracts and isolated compounds are safer for concomitant use with *Poecilia reticulata*. Many authors have reported the safer use of plant-derived larvicidal agents such as *Cuminum cyminum* and *Pimpinella anisum*⁵¹, *Foeniculum vulgare*, *Matricaria chamomilla*⁶², *Atlantia monophylla*¹¹⁵, *Trichodesma indicum*²⁰³ on nontarget organisms such as molluscs, fishes, aquatic insects, and non-vector mosquito species.

The suitability index (SI) or Predator safety factor (PSF), a parameter that measures the suitability of the extract/ isolated compound for integrated vector control was calculated. Comparing, SI of CVA/C1/F16-17/03 and CVA/C1/F16-17/04, CVA/C1/F16-17/04 was more active than the chloroform extract of CVA. Also, lower SI values in comparison to susceptible mosquito species *Anopheles stephensi*, *Aedes*

aegypti narrows the toxic window for dose fixation in formulation¹¹⁵. Considering all these factors, formulation developments proceeded with CHE of CVA.

5.13: Pesticidal formulations

The formulation of CHE of CVA as floating tablets, liquid formulations that emulsify, and wettable powders were selected since they are advantageous for the incorporation of CHE as they enable subsequent release into aquatic habitats.

5.13.1: Tablets- Floating tablet formulation

Mosquito larvae float at the water's surface when they breathe. Floating tablets were formulated to deliver the active extract to the larval floats. The tablets were formulated to inhibit larval respiration with the synergistic larvicidal activity to increase larval mortality. Formulations F1-4 (400 mg each) were punched in a tablet press following the wet granulation method. Natural binders/adjuvants like sodium alginate, xanthum gum, karaya gum, acacia, and tamarind seed kernel gum, were used along with citric acid and sodium bicarbonate were incorporated to induce the floatation¹³⁵.

Fifteen tablet formulations were assessed in the preliminary trials. The FT-IR was then used to analyze the chemical interactions between extract and polymers. No major shift in the peaks indicates the non-reactive nature of the polymers and binding agent with chloroform extract¹³⁵⁻¹³⁷. The four formulations had zero chemical interactions were punched into final form and designated as F1-4. Chemical interactions in the formulations may lead to degradation of actives and hence lowering the larvicidal activity.

The physicochemical characteristics that may alter the stability of the tablets were well within pharmacopeial limits¹³⁶. Tablets were round, green, brown in color, with an average thickness of 6mm and a diameter of 12mm. The weight variation friability and

study were within pharmacopeial limits (of less than 5%)^{120,121}. Low weight variations and hardness test signify consistent preparation, reproducibility, and high strength avoiding problems such as chipping, breaking, swelling etc. HPLC analysis results indicated that the tablets exhibited uniform drug distribution with biomarkers in comparison to the crude CHE of CVA. All the formulations showed satisfactory *in vitro* buoyancy with an insignificant lag time that prolonged drug release up to 38-40 hrs. Formulation F4 had the highest swelling index and was directly proportional to the amount of HPMC K4M, sodium alginate, and xanthum gum and inversely to karaya gum in comparison with other tablet formulations. The slow imbibition of water was due to gum and subsequent diffusion of extract was due to a high percentage of swelling²⁰⁸.

The tablet formulations F1-F4 were screened for larvicidal activity on all the target mosquito species. Formulation F4 was the most effective floating tablet, as all the dosage parameters (flotation, swelling, and drug release profile) were optimized as required for an end user²⁰⁸. A neem-based marketed floating tablet formulation developed by the Indian Institute of Pesticide Formulation Technology had similar properties as we formulated F4 Floating tablets for dengue vector *Aedes*²⁰⁵.

5.13.2: Liquid concentrates

The liquid concentrates are biphasic liquids that can emulsify immiscible ingredients similar to CHE of CVA. The World Health Organization recommends emulsifiable concentrates for treating mosquito larvae in larval habitats. A stable O/W type emulsion with the ideal HLB value of 8-16 was formulated and tested for physicochemical parameters. Gallic acid and sodium benzoate used retard oxidation and microbial contamination²⁰⁹. The average size of the oil globules in the emulsion was $4 \pm 0.56 \mu\text{m}$ and was ideal as globule size distribution for a stable emulsion²⁰⁸⁻¹⁰. The viscosity and

specific gravity of the emulsion were due to gum²¹⁰. The pH of formulations F5-7 was towards the alkaline range to match the pH conditions of the mosquito breeding habitats and not affect the nontarget species²⁰⁸⁻²⁰⁹. Though the drug release profiles, particle size, specific gravity, and viscosity, stability of F5, F6, and F7 were similar, F5 had a better larvicidal activity. F5-7 could still be considered a prototype product for testing liquid concentrates as emulsions in the future.

5.13.3: Wettable powders

Wettable powders are dry formulations that should be premixed into a uniform slurry and then dispersed in water just before application^{146, 214}. For the slurry to be homogeneous, preserving optimal physicochemical conditions is ideal. Particle size and flow characteristics affect spread ability and dissolution. Wettable powder formulations F8-F12 had an optimal moisture content of $\leq 3\%$. The powder characteristics were the pharmacopeial limits of an ideal powder²¹⁵. Upon dissolution, the pH of solutions was neutral to slightly alkaline, similar to the mosquito larval habitats²¹³. Jamnback H et al. reported a similar wettable formulation of methoxychlor and DDT. The results are conclusive that the Wettable formulations are suitable for application at stagnant mosquito breeding sites such as ponds, reservoirs, lakes, etc while for streams/simulated flowing water, a higher dose may be required²¹⁴.

The application of wettable powders could be tested in the future in simulated flowing water conditions. Another important feature of wettable powder is its ease of handling²¹⁶. Researchers have reported that handling wettable powders has lower practical difficulties at large larval breeding sites than that of liquids or tablets²¹⁷.

5.13.2.2: Larvicidal Activity of Developed Formulations

Based on the ease of production and handling, three types of formulations were initiated for the formulation development process from CHE of CVA for larvicidal activity. One of the objectives of our study was to evaluate larvicidal activity of the formulations after all the physicochemical evaluations. Overall, F4 of tablet, F5 of liquid concentrate, and F10 of wettable powder had lower larvicidal LC₅₀ values at 24 hrs. On further observation, it was found that LC₅₀ values of F4 were significantly similar to crude CHE of CVA regardless of dosage form formulated.

For wettable powders, % mortality was higher at 6-12 hrs since the powders in comparison to tablets have a larger surface area. Also, the active CHE was more soluble due to a higher dissolution rate and had significant larvicidal effects in its powder form. In our study, wettable powders and tablets were the best compared to liquid concentrate emulsion. The results are concurrent with the literature, with practical application factors such as ease of handling, application, and dosage measurement²¹⁶.

5.13.4: Stability Studies of Formulations

Selective formulations F4, F5, and F10 that showed the lowest larvicidal LC₅₀ values with good drug release profiles were pursued stability studies at 28°C and 40°C at RH of 70% for 180 days (6 months). The physicochemical parameters of formulations, larvicidal efficacy, and drug release characteristics were recorded for 1, 7, 14, 30, 60, 120, and 180 days as per standard protocols¹²⁵.

No significant changes in the physicochemical properties were observed in tablet formulation F4 and water-dispersible powder formulation F10. In liquid concentrate F6, at 40°C, changes in density and viscosity were observed and may have accounted for higher LC₅₀ values compared to tablets formulation F4 and water-wettable powder formulation F10. Thus, the emulsion formulation began to degrade.

On comparison of the LC₅₀ values of stability-tested formulations F4, F5, F10, at 28°C and 40°C, formulations F4 and F10 had significant larvicidal activity and were more stable even with alterations in humidity and temperature induced²¹⁸.

5.13.5: Herbal Formulations and Synthetic Pesticides in Real World ⁶⁷⁻¹⁵²

The developed herbal formulations are better off than synthetic pesticides, with in terms of several factors such as efficacy, environmental impact, cost, human safety, and long-term sustainability.

Factor	Herbal Formulations	Synthetic Pesticides
Mode of action	Multi modal- larvicidal, oviposition deterrent, and insect growth inhibitor. Similar to standard neem formulation	Directly toxic to pests, affecting nervous system or metabolism
Effectiveness	Effective at medium to large doses of application	Effective at lower doses
Resistance development	Lower risk of resistance due to complex natural compounds	High resistance risk
Toxicity to humans	Generally low toxicity, safer for farmworkers	Can be highly toxic, linked to health issues (neurological, hormonal)
Exposure risks	Low dermal and inhalation risks	High risk of inhalation and skin absorption
Toxicity to humans	Generally low toxicity, safer for farmworkers	Can be highly toxic, linked to health issues (neurological, hormonal)
Cost of production	Can be cost-effective if locally sourced	Expensive due to chemical synthesis
Storage & shelf life	Shorter shelf life, may require cold storage	Long shelf life, easy storage
Application frequency	Frequent applications needed	Fewer applications required
Availability	Limited in some regions	Widely available

5.13.6: Limitations and future prospects of the study¹²⁸⁻¹⁵⁶

To successfully implement herbal mosquito larvicides as a sustainable alternative to synthetic chemical larvicides, a multi-stage approach is necessary. Below is an expanded discussion of key aspects involved.

1. Small-scale field trials serve as preliminary studies to assess the efficacy, feasibility, and environmental interactions of herbal mosquito larvicides before progressing to large-scale deployment. The outcomes include identification of the most effective concentration, application method, initial understanding of non-target effects and degradation rates, assess feasibility of scaleup production.

2. Large-scale field trials validate the findings of small-scale tests under real-world conditions, ensuring effectiveness across diverse ecological and climatic zones, application strategies, long term monitoring and community engagement. These confirm wide-area effectiveness and logistical feasibility, establishment of optimal application intervals for sustainable mosquito control with added advantage of data reports to support regulatory approvals and commercial viability.

3. Long-term ecological impact studies ensures that herbal mosquito larvicides are environmentally sustainable, evaluate their long-term impact on ecosystems and public health, resistance development. This will serve as guide to develop safe-use guidelines, minimize unintended ecological effects, evidence to support long-term sustainability claims, identification of mitigation measures to reduce environmental risks.

4. Integration into pest control programs enables widespread adoption of herbals into existing vector control frameworks, ensuring compatibility with public health strategies including regulatory approval, cost-effectiveness, public awareness, and integration with other methods.

SUMMARY

Based on the literature review, the study was initiated to isolate pesticidal compounds and to formulate eco-friendly formulations from the selected plants “*Cleome viscosa* Linn.” and “*Artocarpus lakoocha* Roxb.,”

The plant selection criteria included ease of availability, literature review, and previous reports on its traditional use with insecticidal properties.

The authentication of plant material and pharmacognostical standardization confirmed the quality and validation of herb materials.

Phytochemical screening of the successive solvent extracts revealed the information on the class of phytochemicals present in each extract so that an optimum method for the isolation of phytoconstituents could be employed.

The physicochemical properties of extracts gave us information related to the nature of extracts, yield-suitability for bulk extraction, and solubility criteria for formulation development.

The three mosquito species (*Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes Aegypti*) were selected for bioactivity-guided isolation of pesticidal compounds, based on criteria that *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* are primary vectors for disease transmission of viral and zoonotic diseases such as dengue, filariasis, and malaria. The order of sensitivity to synthetic pesticides is *Culex quinquefasciatus* << *Anopheles stephensi* << *Aedes Aegypti*. Mosquito collection, culturing, and maintenance were at the NIMR, Bengaluru. The eggs of *Anopheles stephensi* and *Aedes aegypti* and egg rafts of *Culex* were collected in and around Yelahanka, Bengaluru, and allowed to grow in separate enamel pans (30 x 25 x 4cm) of 2 L capacity. Larvae were maintained under a photo regime of 14:10 (L: D) and a

temperature of $27 \pm 2^\circ\text{C}$. The larvae were fed using a 3:1 mixture of dog biscuit powder and dried yeast powder until the 3rd instar larvae developed.

Larvicidal preliminary screening and toxicity study was carried out according to the WHO Pesticide Evaluation Scheme WHO/CDS/WHOPES/GCDPP/ 2005.

The preliminary screening was to determine the activity of extracts and to obtain the concentration range at which the plant extracts are active, while toxicity studies reveal data on the concentrations to avoid future resistance development.

Among the extracts, 150 ppm of CHE of CVA showed the highest 100% mortality as a larvicidal agent at 72 hrs. LC_{50} values of 55.50, 24.25, and 36.86 ppm on *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* was recorded at 24 hrs.

These results are comparable to other authors who have reported similar activity on other insect species. With Artocarpus, PEE had the highest and most significant larvicidal activity compared to other solvent extracts.

Oviposition deterrence activity determines the population dynamics and disease endemics of vector-borne diseases.

10 ppm and 50 ppm of CHE of CVA and PEE of ALL had 100% oviposition deterrence. The results were conclusive that the PEE of ALL induced forced egg retention, the degeneracy of eggs, low hatchability (nonviable eggs), and contact adulticidal effects on the gravid female mosquito in all mosquito species.

Insect growth regulating (IGR) activity resulted in observations of JH analogs by mimicking JH and holding the larvae in as juveniles and/or inducing super moulting effects. The larval period for emergence in the control group is 6-8 days but the extracts prolonged larval period.

The CHE of CVA and PEE of ALL skipped the intermediate stages of development to produce nonviable critters such as tiny immature, sterile, and underdeveloped adults.

with CHE of CVA, the larval period elongated up to 15 days, while with PEE of ALL super-moulting effects by inhibiting the chitin synthesis were observed. Mortality as albino pupae, early molting, and emergence within five days were observed compared to control.

From the results of larvicidal bioassay, juvenile hormone-mimicking activity, and oviposition deterrence of petroleum ether, chloroform, ethanolic, and aqueous extracts, the chloroform extract of aerial parts of CV and petroleum ether extract leaves of AL had multimodal pesticidal activity. The selected plants with multiple modes of insecticidal action indicate that the plant extracts can be formulated/ commercialized. Bioactivity-guided isolation of larvicidal compounds was done to identify marker molecules and to manufacture products. Bioactivity-guided isolation is a systematic approach to finding the biomarkers for *in-vitro/ in-vivo* studies.

BAGI of CHE of CVA resulted in the isolation of 4 compounds viz., CV/C1/F6-8/C2/F1/comp-01 and CV/C1/F6-8/C2/F2/comp-02 (non-larvicidal) and CV/C1/F6-8/C2/F3/comp-03 and CV/C1/F16-17/comp-04 were active for larvicidal activity. At 10ppm, CV/C1/F6-8/C2/F3/comp-03 and CV/C1/F16-17/comp-04 recorded percentage of activity of 60%, 20% at 6 hrs; 80%, 30% at 12 hrs and 100, 60% mortality at 24hrs. After 48 hours, mortality was 100% for both isolated compounds.

Three compounds Viz., ALL/C1/F5/C2/F1-2/comp-01, ALL/C1/F4-5/C2/F3/comp-02 and ALL/C1/F4-5/C2/F4-6/comp-03 were isolated from petroleum ether extract leaves of AL. ALL/C1/F5/C2/F1-2/comp-01 and ALL/C1/F5/C2/F4-6/comp-03 had a maximum larvicidal activity of 50, 30% at 6 hrs; 60%, 40% at 12 hrs and 80, 60% mortality at 24hrs respectively. After 48 hours, mortality was 100% for both isolated compounds.

The isolated compounds were characterized by chromatographic and spectroscopic methods as Compound CV/C1/F6-8/C2/F4/comp-01, a flavonoid glycoside, **isorhamnetin 3-*O*- β -d-glucopyranoside**., Compound CV/C1/F6-8/C2/F4/comp-02, **Quercetin 3-*O*-(2''-acetyl)-glycoside**, Compound CV/C1/F6-8/C2/F4/comp-03, **1- S- [N-(sulonatoxy) but- 3 -nimidoyl] -1 –thio- beta – D glucopyranose or Sinigrin (allyl isothiocyanate)**, CV/C1/F16-17/04, as **5- hydroxy 1, 4 naphthoquinone di-one- 6- beta – D glucopyranose or Juglone-3-D- glucopyranose**, ALL/C1/F4-5/C2/F1-2/comp-01, as a steroidal compound- **Cycloartenol /9 β ,19-cyclo- 24-lanosten-3 β -ol**, ALL/C1/F4-5/C2/F3/comp-02, as 2-arylbenzofurans- **Lakoochin A**, ALL/C1/F4-5/C2/F1-2/comp-03 as **Albafuran B**.

Three compounds were isolated for the first time from *Cleome viscosa* Linn. Viz., **Isorhamnetin 3-*O*- β -d-glucopyranoside**; **1- S- [N-(sulonatoxy) but- 3 -nimidoyl] -1 –thio- beta – D glucopyranose or Sinigrin**; **5- hydroxy 1, 4 naphthoquinone di-one- 6- beta – D glucopyranose Juglone-3-D- glucopyranose**; along with a known compound **Quercetin 3-*O*-(2''-acetyl)-glycoside**.

Similarly, **Cycloartenol**, **Lakoochin A**; and **Albafuran B** have been isolated and identified for the first time from the leaves of *Artocarpus lakoocha* Roxb., even though they were previously reported from the bark and wood.

The Suitability Index or Predator safety factor was used to find the effects of extracts and isolated compounds on non-target species.

Poecilia reticulata, a larvivorous fish was chosen as a non-target species that preys on *Culex*, *Aedes*, and *Anopheles* larvae. The LC₅₀ values observed on *Poecilia reticulata* were much higher than the LC₅₀ values of target mosquito species. The greater the SI value, the greater the safety of non-targets.

SI values for PEE of ALL and its isolated compounds were low despite its significant larvicidal activity. SI of isolated compounds, CV/C1/F16-17/03 > CV/C1/F16-17/04 were greater than the CHE of CV. However, the % yield of CV/C1/F6-8/C2/F4/03 was too low for formulation development. On an overall comparison, SI of ALL and CVA, CVA was more suitable to be formulated.

Hence, the CHE of CVA with compound CV/C1/F6-8/C2/F4/03, was predetermined as a biomarker for larvicidal activity, and the CHE of CVA was used for formulation development. CHE of CVA was formulated as floating tablets, liquid concentrate formulation- -emulsion, and wettable powders based on the advantages of incorporation and its subsequent release into aquatic habitats for vector larval control.

Prototypes F1-4 for floating tablets, F5-7 for liquid concentrates, and F8-12 for wettable powder were produced. F4 of tablets, F5 of liquid concentrate, and F10 with their best physicochemical properties as pesticidal formulations were finalized. Physicochemical properties and drug release profile contributed to the larvicidal activity with significant and lower LC_{50} values at 24 hours compared to CHE.

LC_{50} values of F4, F5, and F10 were closer to CHE even on formulation. In wettable powders, the active CHE was more soluble, and the percentage mortality as larvicidal increased for 6-12 hours. Also, it's noteworthy that the wettable powder has a greater diffusion surface area than tablets and liquid concentrates.

Overall, based on larvicidal activity, wettable powders, and tablets were the best compared to liquid concentrate, with ease of handling, dose measurement, and application, liquid concentrates and tablets are undemanding.

Stability testing of selected formulations F4, F5, and F10 at 28 ± 5 °C/RH of $70 \pm 5\%$, and 40 ± 5 °C/ RH of $70 \pm \%$, were carried out as per standard protocols¹⁸⁵. The testing parameters such as larvicidal activity, drug release characteristics, and physicochemical

parameters of formulations were evaluated at time intervals of 1, 7, 14, 30, 60, 120, and 180 days. No significant changes were observed in the physicochemical properties in tablet formulation F4 and wettable powder formulation F10. While in liquid concentrate formulation F5, changes in density and viscosity with degradation of actives were observed at 40°C may account for lower larvicidal activity and higher LC₅₀ values compared to that of tablets formulation F4 and water wettable powder formulation F10. To conclude on the stability data, the liquid concentrate formulation began to degrade and F4 and F10 were stable. On comparison of LC₅₀ values of stability-tested formulations (F4, F5, and F10), F4 and F10 had significant and stable larvicidal activity and were more stable even with alterations in humidity and temperature. Thus, stable formulations, F4 and F10 can be improvised for scale-up to reap commercial benefits.

On Comparison of Herbal Formulations and Synthetic Pesticides in real world application is that herbal formulations are more environmentally friendly and safer for human health but require frequent applications. Synthetic pesticides are fast-acting and cost-effective; however, they contribute to environmental pollution, health risks, and resistance issues. Integrated Pest Management (IPM), which combines both approaches, is often the best solution for sustainable mosquito control.

Limitations of herbal mosquito Formulations as larvicides

Variability in efficacy with environmental factors such as temperature, water quality, and organic matter can affect the stability and effectiveness of herbal larvicides. Susceptibility of species-specific effects on different mosquito species making uniform control difficult. Another issue is that herbal compounds often tend to degrade faster than synthetic alternatives, reducing their long-term effectiveness.

Standardization and quality control - batch-to-batch variation with differences in plant source, growth conditions, and extraction methods can lead to inconsistent in potency of formulations. Large-scale production and formulation issues on scalability: large-scale production may not be economically viable due to seasonal availability and variable yield of plant sources. Ecological and non-target effects herbal larvicides may affect beneficial aquatic organisms such as fish, amphibians, and insects and also with persistence in water bodies.

Regulatory and approval challenges with the lack of regulatory frameworks and approval pathways compared to synthetic pesticides, long term toxicological study for human and environmental exposure are often insufficient.

Production cost and market acceptance: large-scale extraction and purification of bioactive herbal compounds can be expensive. Also, synthetic larvicides with proven efficacy may still be preferred due to their established market presence and lower costs.

The project addressed the challenges of identification of plants, identification of effective extract, effective dose, standardisation of extract using biomarkers for larvicidal activity, formulation development, quantification of markers in formulation, and its stability studies with future prospects as discussed in the limitations of the study.

CONCLUSION

The plant raw materials were collected from a new and reliable biodiversity-rich geographical source. The collected plant materials are sustainable and can be sourced authentically. Pharmacognostical standardization conferred quality control standards on the plant material used in the study.

Primary vector species- *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* were used as a representative of the family and genera. The multimodal pesticidal activity including larvicidal, Oviposition deterrence, and Juvenile hormone-mimicking- IGR activity was carried out on successive solvent extracts.

Active pesticidal extracts: The chloroform extract of *Cleome viscosa* and the petroleum ether extract of leaves of *Artocarpus lakoocha* had significant ($p < 0.05$) pesticidal activity as larvicidal, oviposition deterrent, and Insect growth regulating agents were the most effective with low LC_{50} values on all three mosquito larvae and adult vector species.

Bioactivity-guided isolation of larvicidal compounds led to the isolation of 6 compounds which were structurally elucidated as: CV/C1/F6-8/C2/F4/comp-01 (flavonoid: iso-rhamnetin 3-*O*- β -D-glucopyranoside), CV/C1/F6-8/C2/F4/comp-02 (Quercetin 3-*O*-(2''-acetyl)-glycoside), CV/C1/F6-8/C2/F4/comp-03 (allyl isothiocyanate: 1- S- [N-(sulfonyl) but- 3-nimidoyl] -1-thio- β -D- glucopyranose or Sinigrin), CV/C1/F16-17/04 (5- hydroxy 1, 4, naphthoquinone di-one- 6- β -D glucopyranose or Juglone-3-D glucopyranose), ALL/C1/F4-5/C2/F1-2/comp-01 (steroid: Cycloartenol), ALL/C1/F4-5/C2/F3/comp-02 (2-aryl benzofuran: Lakoochin A), ALL/C1/F4-5/C2/F1-2/comp-03 (Albafuran B).

Out of which three compounds were isolated for the first time from *Cleome viscosa*

Linn. Viz., isorhamnetin 3-*O*- β -D-glucopyranoside; 1- S- [N-(sulonatoxy) but- 3 - nimidoyl]-1-thio-beta-D glucopyranose or Sinigrin; 5-hydroxy 1,4 naphthoquinone di-one- 6- beta- D glucopyranose Juglone-3-D- glucopyranose; along with known compound Quercetin 3-*O*-(2''-acetyl)-glycoside. Similarly, Cycloartenol, Lakoochin A; and Albafuran B were isolated and identified for the first time from the leaves of *Artocarpus lakoocha* even though they were previously reported from the other parts of the tree like bark and wood.

Larvicidal activity of isolated compound Sinigrin was greater than juglone-o-glucopyranose. Isolated compound Sinigrin was considered as a biomarker for larvicidal activity and chloroform extract was taken up for formulation development in different dosage forms.

Toxicity on non-target organisms: Toxicity (as Suitability Index) was evaluated on *Poecilia reticulata* a larvivorous fish. Chloroform extract and its isolated compounds with higher larvicidal activity and SI were safer than petroleum ether extract of *Artocarpus lakoocha* Roxb.

Herbal Formulations: Chloroform extract with marker compounds was formulated as floating tablets, liquid concentrate, and wettable powders. The formulations with significant physicochemical properties including drug release profiles and larvicidal activity viz., F4, F5, and F10 were subjected to stability studies. Stability studies performed at varied temperatures and humidity did not alter the physicochemical properties including drug release profiles and larvicidal activity of F4 tablets, and F10 wettable powder. However, the formulation of F5-liquid concentrate became unstable due to phase separation. Stable formulations, F4 and F10 can be improvised for scale-up to reap commercial benefits.

All results were subjected to Statistical evaluation using analytical software for calculation with >95% confidence limits and a significance of $p \leq 0.05$.

Herbal formulations and synthetic pesticides in real world application:

Herbal formulations are more environmentally friendly and safer for human health but require frequent applications. Synthetic pesticides are fast-acting and cost-effective; however they contribute to environmental pollution, health risks, and resistance issues. Integrated Pest Management (IPM), which combines both approaches, is often the best solution for sustainable Mosquito control. The project addressed the challenges of identification of plants, identification of effective extract, effective dose, standardisation of extract using biomarkers for larvicidal activity, formulation development, quantification of markers in formulation, and its stability studies.

Future directions for the study

Small scale trails, large scale field trails, long-Term Ecological Impact Studies, integration of herbal formulation in pest control programmes, Regulatory Approval for field application, Integration with other methods by combining herbal larvicides with larval source management (LSM), biological control (e.g., fish that feed on larvae), and insecticide-treated bed nets for a holistic approach.

Global relevance of the findings

The spread of mosquitoes and vector borne diseases is a global challenge faced by developed and developing countries. synthetics pesticides are still the choice in mosquito vector control. Herbal insecticides are being developed by addressing insecticide resistance, reducing environmental harm, health and safety concerns.

The findings of the study support the natural, effective and sustainable mosquito control that can find its way into the real-world application.

Through scientific validation, improved formulations, and policy integration, herbal formulations developed can become a key component in large-scale vector control programs, particularly in regions heavily burdened by mosquito-borne diseases.

The project addressed the challenges of identification of plants, identification of effective extract, effective dose, standardisation of extract using biomarkers for larvicidal activity, formulation development, quantification of markers in formulation, and its stability studies with future prospects as discussed in the limitations of the study.

Finally, the Null hypothesis could be rejected from the significant ($p < 0.05$) and positive outcomes of the tested parameters and the alternate hypothesis could be accepted i.e.,

“Aerial parts of *Cleome viscosa* Linn., and leaves and fruits of *Artocarpus lakoocha* Roxb., are active as pesticidal agents against primary vector species- *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*”.

BIBLIOGRAPHIC REFERENCES

1. Fact sheet: Global Burden of Major Vector-Borne Diseases: World Health Organization. Annex1. [updated 2017 Mar 3]. Available from: <https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases>
2. A Nesibu, Beyene P, W Metiku, Dejene T, Mesfin L. A study of the status of *A. tenebrosus* (Donitz, 1902) in the transmission of malaria in Sille, southern Ethiopia. *Ethiopian J of Health Development*. 1998 Jan 1;12:75-80.
3. Tyagi BK, A. Munirathinam, Venkatesh A. A catalog of Indian mosquitoes. *Intern. J of Mosquito Res*. 2015 Jun 1;2(2):50-97.
4. Centers for Disease Control and Prevention. Mosquito life cycle *Aedes aegypti* [Internet]. Available from: <https://www.cdc.gov/dengue/resources/factSheets/MosquitoLifecycleFINAL.pdf>
5. Pingen M, Bryden Steven R, Pondeville E, Schnettler E, Kohl A, Merits A, et al. Host Inflammatory Response to Mosquito Bites Enhances the Severity of Arbovirus Infection. *Immunity* [Internet]. 2016 Jun [cited 2019 Oct 24];44(6):1455-69.
6. Vector-borne diseases [Internet]. www.who.int. Available from: <https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases#:~:text=It%20causes%20an%20estimated%20219>
7. Wilson AL, Courtenay O, Kelly-Hope LA, Scott TW, Takken W, Torr SJ, et al. The importance of vector control for the control and elimination of vector-borne diseases. Barrera R, editor. *PLOS Neglected Tropical Diseases* [Internet]. 2020 Jan 16;14(1):e0007831.
8. Mutero CM, Schlodder D, Kabatereine N, Kramer R. Integrated vector management for malaria control in Uganda: Knowledge, Perceptions and Policy development. *Malaria J* [Internet]. 2012 Jan 14 [cited 2020 Oct 27];11:21.
9. Briggs JD. Principles of Integrated Control of Disease Vectors. *American zoologist*. 1970 Nov 1;10(4):567-71.
10. Garnham C. Zoonoses or infections common to man and animals. 1958 Apr 1;61(4):92-4.
11. Division of Vector-Borne Diseases (DVBD) | Division of Vector-Borne Diseases. NCEZID CDC [Internet]. www.cdc.gov. 2023. Available from: <https://www.cdc.gov/ncezid/dvbd/index.html#:~:text=Who%20we%20are>

12. Senthil-Nathan S. A Review of Resistance Mechanisms of Synthetic Insecticides and Botanicals, Phytochemicals, and Essential Oils as Alternative Larvicidal Agents Against Mosquitoes. *Frontiers in Physiology*. 2020 Feb 25;10.
13. Takeshi N, Mori A, Toshinori K, Iwata Y, Osamu H, Harada S, et al. An amino acid substitution attributable to insecticide-insensitivity of acetylcholinesterase in a Japanese encephalitis vector mosquito, *Culex tritaeniorhynchus*. *Biochemical and Biophysical Research Communications*. 2004 Jan 1;313(3):794-801.
14. Rajashekar Y, Raghavendra A, Bakthavatsalam N. Acetylcholinesterase Inhibition by Biofumigant (Coumaran) from Leaves of *Lantana camara* in Stored Grain and Household Insect Pests. *BioMed Research Intern*. 2014;2014:1-6.
15. Rawani A, Ghosh A, Chandra G. Mosquito larvicidal activities of *Solanum nigrum* L. leaf extract against *Culex quinquefasciatus* Say. *Parasitology Res*. 2010 Jul 29;107(5):1235-40.
16. Cheng S. Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae. *Bioresource Technol*. 2003 Aug;89(1):99-102.
17. Hemingway J, Ranson H. Insecticide Resistance in Insect Vectors of Human Disease. *Annual Review of Entomology* [Internet]. 2000 Jan [cited 2019 Aug 31];45(1):371-91.
18. Arthur FH. Grain protectants: Current status and prospects for the future. *J of Stored Products Res*. 1996 Oct;32(4):293-302.
19. Wesseling C, Keifer M, Ahlbom A, McConnell R, Moon JD, Rosenstock L, et al. Long-term Neurobehavioral Effects of Mild Poisonings with Organophosphate and n-Methyl Carbamate Pesticides among Banana. *Intern. J of Occupational and Environmental Health*. 2002 Jan;8(1):27-34.
20. Young JG, Eskenazi B, Gladstone EA, Bradman A, Pedersen L, Johnson C, et al. Association Between In Utero Organophosphate Pesticide Exposure and Abnormal Reflexes in Neonates. *NeuroToxicology*. 2005 Mar;26(2):199-209.
21. Damalas CA, Eleftherohorinos IG. Pesticide Exposure, Safety Issues, and Risk Assessment Indicators. *Intern. J of Environmental Research and Public Health* [Internet]. 2011 May 6;8(5):1402-19.
22. Rivero A, Vézilier J, Weill M, Read AF, Gandon S. Insecticide Control of Vector-Borne Diseases: When Is Insecticide Resistance a Problem? Manchester M, editor. *PLoS Pathogens* [Internet]. 2010 Aug 5;6(8):e1001000.

23. Dhiman R, Kumar G, Ojha V. Insecticide susceptibility status of malaria vectors in Himachal Pradesh, India. *J of Vector Borne Diseases*. 2022;59(3):236-240.
24. Resistance of vectors of disease to pesticides. Fifth report of the WHO Expert Committee in Vector Biology and Control. 1980 Jan 1;655:1-82.
25. Ajaiyeoba EO, Sama W, Essien EE, Olayemi JO, Olusegun Ekundayo, Walker TM, et al. Larvicidal Activity of Turmerone-Rich Essential Oils of *Curcuma longa*. Leaf and Rhizome from Nigeria on *Anopheles gambiae*. *Pharmaceutical Biology*. 2008 Jan 1;46(4):279-82.
26. Lale A, Kulkarni D. A Mosquito Repellent Karanj Kunapa from *Pongamia pinnata*. 2010; 14(2): 207-211.
27. Alice J, Sujeetha RP. Larvicidal activity of Neem and Palmarosa oil against a filarial vector *Culex quinquefasciatus*. *J Ecotoxicol Environmental Monitor*. 2001;11(2):157-58.
28. Anuradha V, Lakshmi T, Sakthivadivel M, Danial T. Effect of certain plant extracts against the fourth instar larvae of filarial vector *Culex quinquefasciatus*. *J Ecobiol*. 2000;12(2): 93-98.
29. Lorenz LM, Maia MF, Moore SJ. The use of plants in vector control. *Biological and Environmental Control of Disease Vectors*. 2013; May: 42-66.
30. Grdisa M, Grsic K. Botanical Insecticides in Plant Protection. *Agric. Conspec. Sci*. 2013;78:85-93.
31. Sharma P, Mohan L, Srivastava CN. Phytoextract-induced developmental deformities in malaria vector. *Bioresource Technol*. 2006 Sep;97(14):1599-604.
32. Kassir JT, Mohsen MZ, Mehdi NS. Toxic effects of limonene against *Culex quinquefasciatus* Say larvae and its interference with oviposition. *Anzeiger für Schädlingkunde, Pflanzenschutz, Umweltschutz*. 1989;62(1):1921.
33. Choochote W, Kanjanapothi D, Panthong A, Taesotikul T, Jitpakdi A, Chaithong U, Pitasawat B. Larvicidal, adulticidal and repellent effects of *Kaempferia galanga*. *Southeast Asian J Trop Med Public Health*. 1999 Sep;30(3):470-6.
34. Azmi MA, Naqvi SNH, Ahmad I, Tabassum R, Anbreen B. Toxicity of neem leaves extract (NLX) compared with malathion (57 E.C.) against late 3rd instar larvae of *Culex fatigans* (Wild Strain) by WHO method. *Turk J Zool*. 1998;22(3):213-8.
35. Senthil NS, Kalaivani K, Murugan K. Effects of neem limonoids on the malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae). *Acta Trop*. 2005;96:47-55

36. Isman MB. Neem and other Botanical insecticides: Barriers to commercialization. *Phytoparasitica*. 1997;25:339-44
37. Mulla MS, Schaefer CH. Developing Better Larvicides. *California Agric*. 1980;
38. Kamaraj C, Bagavan A, Elango G, Zahir AA, Rajakumar G, Marimuthu S, Santhoshkumar T, Rahuman A. Abdul Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* & *Culex tritaeniorhynchus*. *The Indian J of Med Res*. 2011;134(1):101-6.
39. Shunying Z, Yang Y, Huaidong Y, Yue Y, Guolin Z. Chemical composition and antimicrobial activity of the essential oils of *Chrysanthemum indicum*. *J Ethnopharmacol*. 2005 Jan 4;96(1-2):151-8.
40. Yenesew A, Derese S, Midiwo JO, Heydenreich M, Peter MG. Effect of rotenoids from the seeds of *Millettia dura* on larvae of *Aedes aegypti*. *Pest Manag Sci*. 2003 Oct;59(10):1159-61. doi: 10.1002/ps.740. PMID: 14561074.
41. Maia MF, Moore SJ. Plant-based insect repellents: a review of their efficacy, development and testing. *Malar J*. 2011;10 (1).
42. Vats S. Larvicidal activity and in vitro regulation of rotenoids from *Cassia tora* L. *3 Biotechnol*. 2018 Jan;8(1):13.
43. Vasconcelos J, Santiago G, Lima J, Mafezoli J, Lemos T, Silva F, Lima M, Pimenta A, Braz-Filho R, Arriaga A, Cesarin SD. Rotenoids from *Tephrosia toxicaria* with larvicidal activity against *Aedes aegypti*, the main vector of dengue fever. *Química Nova*. 2011;35:1097-100.
44. Ekapratiwi Y, Rachmadiva, Virgine K, Fauzantoro A, Gozan M, Jufri M. The effect of tobacco extracts based bio larvicide emulsion formulation against *Aedes aegypti* larvae. *AIP Conference Proceedings*. 2019; 2092. 030009. 10.1063/1.5096713.
45. Chen Y, Yang J, Zhang G., et al. Larvicidal activity and microencapsulation of tobacco (*Nicotiana tabacum*) extract on *Malacosoma neustria* testacea larvae. *J. For. Res*. 2021;32:1763-73.
46. Kasiemobi O, Mauel B, Membere. (2014). Larvicidal effect of aqueous leaf extract of tobacco [*Nicotiana tabacum*] on the third instar larvae of *Musca domestica*. *IOSR J of Agric. and Veterinary Sci*. 2014;7:35-40.
47. Banerji B, Nigam S. Wood constituents of *Meliaceae*: a review. *Fitoter* 1984; 55: 3-36.

48. Senthil Nathan S. A Review of Resistance Mechanisms of Synthetic Insecticides and Botanicals, Phytochemicals, and Essential Oils as Alternative Larvicidal Agents Against Mosquitoes. *Front Physiol.* 2020; Feb 25;10:1591.
49. Chandler D, Bailey AS, Tatchell GM, Davidson G, Greaves J, Grant WP. The development, regulation, and use of biopesticides for integrated pest management. *Philos Trans R Soc Lond B Biol Sci.* 2011; Jul 12;366(1573):1987-98.
50. Benelli G, Pavela R, Petrelli R, Cappellacci L, Canale A, Senthil NS, et al. Not just popular spices! essential oils from *Cuminum cyminum* and *Pimpinella anisum* are toxic to insect pests and vectors without affecting non-target invertebrates. *Indus. Crop. Prod.* 2018;124: 236-243.
51. Malviya, Neelesh, Malviya. Bioassay-guided fractionation-an emerging technique that influences the isolation, identification, and characterization of lead phytomolecules. *Intern. J of Hospital Pharmacy.* 2017;2(5):1-6. 10.28933/ijhp-2017-07-0901.
52. Neghabi HM, Aliahmadi A, Taheri MR, Ghassempour A, Irajian G, Rezadoost H, Feizabadi MM. A bioassay-guided fractionation scheme for characterization of new antibacterial compounds from *Prosopis cineraria* aerial parts. *Iran J Microbiol.* 2016;8(1):1-7.
53. Singh J, Kumar A, Sharma A. Bioactivity Guided Fractionation of Ethanol Extract of *Caesalpinia digyna* Rottler Roots. *Pharmacognosy J.* 2016;8(2):165-167.
54. Joshua JK, Daniel AT, Joseph ME, Huzefa AR, Nicholas HO, Olav MK, Nadja BC. Biochemometrics for Natural Products Research: Comparison of Data Analysis Approaches and Application to Identification of Bioactive Compounds. *Pharmacognosy J.* 2016;8(2):376-386
55. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Afr J Tradit Complement Altern Med.* 2011;8(1):1-10.
56. Li S, Han Q, Qiao C, Song J, Lung Cheng C, Xu H. Chemical markers for the quality control of herbal medicines: An overview. *Chin Med.* 2008;28;3:7.
57. Narayanankutty A, Kuttithodi AM, Alfarhan A, Rajagopal R, Barcelo D. Chemical Composition, Insecticidal and Mosquito Larvicidal Activities of Allspice (*Pimenta dioica*) Essential Oil. *Molecules.* 2021 Nov 5;26(21):6698.

58. Mohan L, Sharma P, Srivastava CN. Evaluation of *Solanum xanthocarpum* extracts as mosquito larvicides. *J Environ Biol.* 2005 Jun;26(2 Suppl):399-401.
59. Milugo TK, Tchouassi DP, Kavishe RA, Dinglasan RR and Torto B (2021) Naturally Occurring Compounds With Larvicidal Activity Against Malaria Mosquitoes. *Front. Trop. Dis.* 2021;2:718804.
60. Rawani A, Ray AS, Ghosh A, Sakar M, Chandra G. Larvicidal activity of phytosteroid compounds from leaf extract of *Solanum nigrum* against *Culex vishnui* group and *Anopheles subpictus*. *BMC Res Notes.* 2017 Mar 23;10(1):135.
61. Al-Mekhlafi FA, Abutaha N, Al-Doaiss AA, Ahmed Al-Keridis L, Alsayadi AI, Ali El Hadi Mohamed R, Wadaan MA, Elfaki Ibrahim K, Al-Khalifa MS. Target and non-target effects of *Foeniculum vulgare* and *Matricaria chamomilla* combined extract on *Culex pipiens* mosquitoes. *Saudi J Biol Sci.* 2021 Oct;28(10):5773-5780.
62. Maheswaran R, Ignacimuthu S. A novel herbal formulation against dengue vector mosquitoes *Aedes aegypti* and *Aedes albopictus*. *Parasitol Res.* 2012 May;110(5):1801-13.
63. Chattopadhyay A, Shaw V, Mukherjee P, Ghosh S, Banerjee P. Development of Plant-Based Larvicide and Herbal Mosquito Repellent Fast Card with Reference to Identification of the Functional Bioactive Compounds Effective Against *Culex* Mosquito. 2021;1-9.
64. Piplani M, Bhagwat DP, Singhvi G, Sankaranarayanan M, Balana-Fouce R, Vats T, Chander S. Plant-based larvicidal agents: An overview from 2000 to 2018. *Exp Parasitol.* 2019 Apr;199:92-103.
65. Narasimman M, Natesan V, Mayakrishnan V, Rajendran J, Venkatesan A, Kim SJ. Preparation and Optimization of Peppermint (*Mentha pipertia*) Essential Oil Nanoemulsion with Effective Herbal Larvicidal, Pupicidal, and Ovicidal Activity against *Anopheles stephensi*. *Curr Pharm Biotechnol.* 2022;23(11):1367-1376.
66. Singh VK, Singh RK, Mishra B, Singh D. Formulation and evaluation of eco-friendly handmade herbal mosquito repellent cone. *Intern. J of Pharmaceutics and Drug Analysis.* 2021 Dec 31;9(4):230-5.
67. Caius JF. The medicinal and poisonous plants of India. Scientific publishers. India. 2003; 273 p.
68. William D, Warder CJH, David H. *Pharmacographica Indica*. Jayed press. New Delhi. 1890; 1:131-32 p.

69. Nadkarni KM, Nadkarni AK. Indian Materia Medica. Popular Prakashan, Bombay. 1976; 1:351-52 p.
70. Kirtikar KR, Basu BD. Indian medicinal plants. Periodical Experts, New Delhi. 1975; 1:183-85 p.
71. Keshavmurthy KR. Medicinal plants of Karnataka. Karnataka Forest Department. Graphic typesetters. Bengaluru. 1994; 91 p.
72. Mahato SB, Pal BC, Kawasaki T, Miyahara K, Tanaka O, Yamasaki K. Structure of cleomilide, a novel Diterpene lactone from *Cleome icosandra* Linn. American chemical Society. 1979; 101:4720-23.
73. Kosela S, Emilo LG, Philip RJ, Brian WS, Allan HW. Unsaturated cembrane acids from *Cleome viscosa* L. (Capparidaceae). Australian J of Chemistry. 1985; 38:1365-70
74. Ray AB, Chattopadyay SK, Sandeep K. Structures of cleomiscosins, coumarinolignoids of *Cleome viscosa* seeds. Tetrahedron. 1985; 41:209-14.
75. Upadhyay R. *Cleome viscosa* Linn: A natural source of pharmaceuticals and pesticides. Intern. J of Green Pharmacy. 2015;9:71-85.
76. Singh AK, Srivastava GN, Abraham Z, Gupta MM. Dictionary of Indian Medicinal Plants. Central Institute of Medicinal and Aromatic Plants. Vap enterprises. Lucknow. 1992; 141 p.
77. Ba NM, Sawadogo F, Dabire-Binso CL, Drabo I, Sanon A. Insecticidal activity of three plants extracts on the cowpea pod sucking bug, *Clavigralla tomentosicollis*, STAL (Hemiptera: Coreidae). Pak J Biol Sci. 2009 Oct 1;12(19):1320-4.
78. Krishnappa K, Elumalai K. Mosquitocidal properties of *Basella rubra* and *Cleome viscosa* against *Aedes aegypti* (Linn.) (Diptera: Culicidae). Eur Rev Med Pharmacol Sci. 2013 May;17(9):1273-7.
79. Chadak RR, Bhairat NK, Devdhe SJ, Majmundae HF. In vitro evaluation of anthelmintic potential of leaves of *Cleome viscosa*. Intern. J Pharmaceutical Sci. Review and Res. 2010;5(3):77-79.
80. Khare CP. Indian Medicinal Herbs: An illustrated dictionary. Springer-Verlag Berlin/ Heidelberg. 2007; 66 p.
81. Anonymous. Wealth of India Raw materials and industrial products. CSIR. New Delhi. 1985; 1:453, 455 p.

82. Jagtap UB, Bapat VA. Artocarpus: A review of its traditional uses, Phytochemistry and Pharmacology. J of Ethnopharmacology. 2010;129(2):142-166.
83. Chatterjee BP, Ahmed H, Chowdhury S. Further characterization of Artocarpus lakoocha lectin (Artocarpin) purified using Rivanol. Carbohydrate Res. 1988;180(1):97-110.
84. Wongkham S, Wongkham C, Boonsiri P, Simasathiansophon S, Trisonthi C, Atisook K. Isolectins from seeds of Artocarpus lakoocha. Phytochemistry. 1995 Nov;40(5):1331-4.
85. Maneechai S, De-Eknamkul W, Umehara K, Noguchi H, Likhitwitayawuid K. Flavonoid and stilbenoid production in callus cultures of Artocarpus lakoocha. Phytochemistry. 2012 Sep;81:42-9.
86. Rakesh KN, Dileep N, Syed J, Prashith TR, Vinayaka KS, Noor NAS. Inhibitory Effect of Cow Urine Extracts of Selected Plants against Pathogens Causing Rhizome Rot of Ginger. Sci., Technol. and Arts Res. 2013;2(2):92-96.
87. Shailendra KMB, Rakesh KMC, Bharath AC, Vinod KHR, Prashith KTR, Nandini KC, Rakshitha MN, Raghavendra HL. Screening of selected biological activities of Artocarpus lakoocha Roxb (Moraceae) fruit pericarp. J of Basic and Clinical Pharmacy. 2011;1:239-45.
88. Saowakon N, Tansatit T, Wanichanon C, Chanakul W, Reutrakul V, Sobhon P. Fasciola gigantica: anthelmintic effect of the aqueous extract of Artocarpus lakoocha. Exp Parasitol. 2009 Aug;122(4):289-298.
89. Safia Z, Aoumeur B, Nabila S, Nesrine C. Chemical composition and larvicidal activity of Algerian Foeniculum vulgare seed essential oil. Arabian J of Chemistry. 2014; 7(4):480-485.
90. Sivakumar R, Jebanesan A, Govindarajan M, Rajasekar P. Larvicidal and repellent activity of tetradecanoic acid against Aedes aegypti (Linn.) and Culex quinquefasciatus (Say.) (Diptera: Culicidae). Asian Pac J Trop Med. 2011 Sep;4(9):706-10.
91. Govindarajan M, Mohan R. Mosquito larvicidal properties of Impatiens balsamina (Balsaminaceae) against Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae) J of Coastal Life Medicine. 2014; 2(3):222-24
92. Mousumi K, Anjali R, Goutam C. Evaluation of Mosquito Larvicidal Activities of Seed Coat Extract of Cassia sophera L. J of Mosquito Res. 2013; 3(11):76-8.

93. Rajamma AJ, Sonal D, Sateesha SB. Pharmacognostic Evaluation and Larvicidal Activity of Selected Three *Ocimum* Species. *Pharmacologyonline*. 2010;1:789-96
94. Rajkumar S, Jebanesan A. Larvicidal and oviposition activity of *Cassia obtusifolia* Linn (Family: Leguminosae) leaf extract against malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitol Res*. 2009 Jan;104(2):337-40.
95. Warikoo R, Kumar S. Oviposition altering and ovicidal efficacy of root extracts of *Argemone mexicana* against dengue vector, *Aedes aegypti* (Diptera: Culicidae). *J of Entomology and Zoology Studies*. 2014; 2(4):11-17.
96. Xue RD, Ali A, Barnard DR. Effects of forced egg-retention in *Aedes albopictus* on adult survival and reproduction following application of DEET as an oviposition deterrent. *J Vector Ecol*. 2005 Jun;30(1):45-8.
97. Swathi S, Murugananthan G, Ghosh SK. Oviposition deterrent activity from the ethanolic extract of *Pongamia pinnata*, *Coleus forskohlii*, and *Datura stramonium* leaves against *Aedes aegypti* and *Culex quinquefasciatus*. *Pharmacogn Mag*. 2010 Oct;6(24):320-2.
98. Rajkumar S, Jebanesan A. Oviposition deterrent and skin repellent activities of *Solanum trilobatum* leaf extract against the malarial vector *Anopheles stephensi*. *J Insect Sci*. 2005;5:15.
99. Smykal V, Daimon T, Kayukawa T, Takaki K, Shinoda T, Jindra M. Importance of juvenile hormone signaling arises with competence of insect larvae to metamorphose. *Dev Biol*. 2014 Jun 15;390(2):221-30.
100. Hadidy, D.E., El Sayed, A.M., Tantawy, M.E. et al. Larvicidal and repellent potential of *Ageratum houstonianum* against *Culex pipiens*. *Nature portfolio Sci Rep*. 2022;12(1):21410.
101. Tunaz H, Uygun N. Insect Growth Regulators for Insect Pest Control. *Turk J Agric For*. 2004;28(6):377-387.
102. Arivoli S, Samuel T. Larvicidal and adult emergence inhibition activity of *Abutilon indicum* (Linn.) (Malvaceae) leaf extracts against vector mosquitoes (Diptera: Culicidae). *J of Biopesticides*. 2011;4(1):27-35.
103. Ferdinand MT, Josphat CM, Rostand MN, Regina C. Mosquito larvicidal activity of alkaloids from *Zanthoxylum lemairei* against malaria vector *Anopheles gambiae*. *Pesticide Biochemistry and Physiology*. 2011;99:82-85.

104. Innocent E, Joseph CC, Gikonyo NK, Moshi MJ, Nkunya MH, Hassanali A. Mosquito larvicidal constituents from *Lantana viburnoides* sp *viburnoides* var *kisi* (A. rich) Verdc (Verbenaceae). *J Vector Borne Dis*. 2008 Sep;45(3):240-4.
105. Rahuman AA, Gopalakrishnan G, Venkatesan P, Geetha K, Bagavan A. Mosquito larvicidal activity of isolated compounds from the rhizome of *Zingiber officinale*. *Phytother Res*. 2008 Aug;22(8):1035-9.
106. Jayaraman M, Senthilkumar A, Venkatesalu V. Evaluation of some aromatic plant extracts for mosquito larvicidal potential against *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles stephensi*. *Parasitol Res*. 2015 Apr;114(4):1511-8.
107. Elumalai D, Hemavathi M, Hemalatha P, Deepaa CV, Kaleena PK. Larvicidal activity of catechin isolated from *Leucas aspera* against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol Res*. 2016 Mar;115(3):1203-12.
108. Kim MG, Jeon JH, Lee HS. Larvicidal activity of the active constituent isolated from *Tabebuia avellanedae* bark and structurally related derivatives against three mosquito species. *J Agric Food Chem*. 2013 Nov 13;61(45):10741-5.
109. Subramanian A, Rajasingh RST. Larvicidal Activity of *Citrullus colocynthis* (L.) Schrad (Cucurbitaceae) isolated fractions against *Aedes aegypti* (L.), *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae). *Indian J. of Applied Res*. 2015;5(8):97-101.
110. Perumalsamy H, Jang MJ, Kim JR, Kadarkarai M, Ahn YJ. Larvicidal activity and possible mode of action of four flavonoids and two fatty acids identified in *Milletia pinnata* seed toward three mosquito species. *Parasit Vectors*. 2015 Apr 19;8:237.
111. Innocent E, Joseph CC, Gikonyo NK, Moshi MJ, Nkunya MH, Hassanali A. Mosquito larvicidal constituents from *Lantana viburnoides* sp *viburnoides* var *kisi* (A. rich) Verdc (Verbenaceae). *J Vector Borne Dis*. 2008 Sep;45(3):240-4.
112. Abdul R, Geetha G, Venkatesan P, Kannappan G, Bagavan A. Mosquito Larvicidal Activity of Isolated Compounds from the Rhizome of *Zingiber officinale*. *Phytotherapy Res*. 2008;22:1035-39.
113. Sivagnaname N, Kalyanasundaram M. Laboratory evaluation of methanolic extract of *Atlantia monophylla* (Family: Rutaceae) against immature stages of mosquitoes and non-target organisms. *Mem Inst Oswaldo Cruz*. 2004 Feb;99(1):115-8.

114. Govindarajan M., Rajeswary M., Hoti S.L. Clerodendrum chinense-mediated biofabrication of silver nanoparticles: mosquitocidal potential and acute toxicity against non-target aquatic organisms. J Asia-Pacific Entomol. 2016;19:51-58.
115. Dua VK, Pandey AC, Raghavendra K, Gupta A, Sharma T, Dash AP. Larvicidal activity of neem oil (*Azadirachta indica*) formulation against mosquitoes. Malar J. 2009 Jun 8;8:124.
116. Yupha R, Yuwadee T, Narumon K, Nuttanan S, Chamnarn A, Aumpol M. Formulation of tablets from the crude extracts of *Rhinacanthus nasutus* (Thai local plant) against *Aedes aegypti* and *Culex quinquefasciatus* Larvae: A Preliminary Study. Southeast Asian J of Tropical Medicine and Public Health. 2006;37(2):265-271
117. Cetin H, Erler F, Yanikoglu A. Larvicidal activity of a botanical natural product, AkseBio2, against *Culex pipiens*. Fitoterapia. 2004 Dec;75(7-8):724-8.
118. Santhoshkumar T, Rahuman AA, Rajakumar G, Marimuthu S, Bagavan A, Jayaseelan C, Zahir AA, Elango G, Kamaraj C. Synthesis of silver nanoparticles using *Nelumbo nucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors. Parasitol Res. 2011 Mar;108(3):693-702.
119. Courtesy: <https://www.arbico-organics.com/product/natural-dt-tablets/pest-solver-guide-mosquitoes>
120. Alyahya HS. Comparative study of three herbal formulations against dengue vectors *Aedes aegypti*. Saudi J Biol Sci. 2023 Jun;30(6):103651.
121. Jack ER, Nancy AR. Insect Pest Management: Techniques for Environmental Protection. Lewis publishers. 1999; 7-8 p.
122. Robert L. Zimdahl, Chapter 17 - Herbicide Formulation, Editor(s): Robert L. Zimdahl, Fundamentals of Weed Sci. (Fifth Edition), Academic Press, 2018, 501-509 p.
123. World Health Organization, Basic Tests For Drugs: Pharmaceutical Substances, Medicinal Plant Materials And Dosage Forms/22 May 2021/Technical document
124. World Health Organization, Quality control methods for medicinal plant materials, WHO/ PHARM/ 92.559, 1998; 04-46 p.
125. Iyengar MA, Pharmacognosy of Powdered Crude Drugs, 5th edition, Manipal Press Limited, Manipal. 1997; 21-31.

126. Guidelines for laboratory and field testing of mosquito larvicides. WHO Pesticide Evaluation Scheme. WHO/CDS/WHOPES/GCDPP/2005; 13 p.
127. Bajpai VK, Majumder R, Park JG. Isolation and purification of plant secondary metabolites using column-chromatographic technique. *Bangladesh J of Pharmacology*. 2016;11(4):844-848.
128. Venkatesh U, Javarasetty C, Murari SK. Purification and fractional analysis of methanolic extract of *Wedelia trilobata* possessing apoptotic and anti-leukemic activity. *Afr J Tradit Complement Altern Med*. 2017 Mar 1;14(3):167-174.
129. Krishna ANV, Kumar P. Column Chromatography Purified Phytochemicals Identified in *Phragmites vallisneria* Leaf Ethanolic Extract. *American J of Adv. Drug Delivery*. 2013;1(5):611-627.
130. Isyaku I, Bello IA, Ndukwe IG, Kizito IG. Isolation and Characterization of Nonanoic Acid from Ethyl Acetate Extract of *Adenodolichos paniculatus* (hua) Hutch. & Dalz (Fabaceae). 2010;5(3):337-342.
131. Ha YR, Yeom E, Ryu J, Lee SJ. Three-dimensional structures of the tracheal systems of *Anopheles sinensis* and *Aedes togoi* pupae. *Sci Rep*. 2017 Mar 13;7:44490.
132. Vargas WG, Jimenez KV, Londono GC, Yepes, FJ. Novel larvicide tablets of *Bacillus thuringiensis* var. *israelensis*: Assessment of larvicidal effect on *Aedes aegypti* (Diptera: Culicidae) in Colombia. *Biomédica*. 2018; 38(2):95-105.
133. Gharti K, Thapa P, Budhathoki U, Bhargava A. Formulation and in vitro evaluation of floating tablets of hydroxypropyl methylcellulose and polyethylene oxide using ranitidine hydrochloride as a model drug. *J Young Pharm*. 2012 Oct;4(4):201-8.
134. Kiran RS, Shekar BC, Babu BN. Preparation and evaluation of floating tablets by nonaqueous wet granulation method of Famotidine. *Intern. J of Pharmaceutical Sci*. 2011;3(1):936-46.
135. Ram HN, Lachake P, Kaushik U, Shreedhara CS. Formulation and evaluation of floating tablets of licorice extract. *Pharmacognosy Res*. 2010 Sep;2(5):304-8.
136. Al-Solami HM. Larvicidal efficacy of three sustained insecticides against *Culex pipiens* larvae. *Intern. J of Mosquito Res*. 2019;6(3): 27-31.
137. Bamiro OA, Eke CP, Alayo MA. The Emulsifying Properties of *Terminalia randii* Baker F. Gum in Castor Oil and Liquid Paraffin Emulsions. *Nigerian J of Pharmaceutical Res*. 2016;12(2):155-162.

138. Amalesh S, & Ojha D, Mukherjee B. Stability analysis of primary emulsion using a new emulsifying agent gum odina. *Natural Sci.* 2010; 2(5):494-505.
139. Shekarforoush E, Mirhosseini H, Sarker MI, Gazali HM, Muhamad K, Samaram S. Soy Protein-Gum Karaya Conjugate: Emulsifying Activity and Rheological Behavior in Aqueous System and Oil in Water Emulsion. *J Am Oil Chem Soc.* 2016;93,1-10.
140. Joseph J, Kanchalochana SN, Rajalakshmi G, Hari V, Durai RD. Tamarind seed polysaccharide: A promising natural excipient for pharmaceuticals. *Int J Green Pharm* 2012;6:270-8.
141. Ravi K, Sachin RP, Patil MB, Mahesh SP, Mahalaxmi R. Isolation and evaluation of the emulsifying properties of tamarind seed polysaccharide on castor oil emulsion. *Der Pharmacia Lettre.* 2010;2(1):518-527.
142. Hadapad A, Hire R, Vijayalakshmi N, Dongre T. Sustained-release biopolymer based formulations for *Bacillus sphaericus* Neide ISPC-8. *J of Pest Sci.* 2011;84. 249-55.
143. Derua YA, Kweka EJ, Kisinza WN, Githeko AK, Mosha FW. Bacterial larvicides used for malaria vector control in sub-Saharan Africa: review of their effectiveness and operational feasibility. *Parasites and Vectors.* 2019;12:426:1-18.
144. Sallehudin S, John J, Abdul RS, Hashim Y, Busparani V, Robiah M. Evaluation of Bactimos wettable powder, granules and briquets against mosquito larvae in Malaysia, *Acta Tropica.* 1990;47(4):189-195.
145. Ajeesh TP, Adarsh TP, Kumuthakallavalli R, Raj V, Juliet S, Shobha T et al. Physico-chemical evaluation and biochemical quantification of crude drug powder (stem) of *Chassalia curviflora* (Wall. ex Kurz.) Thwaites; A folk medicinal plant. *J of Pharmacognosy and Phytochemistry.* 2014;3(4):121-124.
146. Farooq S, Rather SA, Gull A, Ganai SA, Masoodi FA, Wani SM et al. Physicochemical and nutraceutical properties of tomato powder as affected by pretreatments, drying methods, and storage period. 2020;23(1)797-808.
147. Liyanage CD, Pieris M. A Physico-Chemical Analysis of Coconut Shell Powder, *Procedia Chemistry.* 2015;16:222-28.
148. ICH Harmonised Tripartite Guideline Stability Testing for New Dosage Forms. Annex to the ICH Harmonised Tripartite Guideline on Stability Testing for New Drugs and Products Q1C, November 1996; accessed on 15 November 2019.

149. ICH Harmonised Tripartite Guideline Stability Testing: Photostability Testing Of New Drug Substances And Products Q1B, November 1996. accessed on 15 November 2019.
150. Mathew A, Rosina A. ICH guidelines for the stability. Conference: Ich Guidelines for Stability Testing of Drugs, Baharain. 2019.
151. Byard RW. Lethal Dengue Virus Infection: A Forensic Overview. *Am J Forensic Med Pathol.* 2016 Jun;37(2):74-8
152. Wilson AL, Courtenay O, Kelly-Hope LA, Scott TW, Takken W, Torr SJ, Lindsay SW. The importance of vector control for the control and elimination of vector-borne diseases. *PLoS Negl Trop Dis.* 2020 Jan 16;14(1):e0007831.
153. Chowdhury R, Chowdhury V, Faria S, Islam S, Maheswary NP, Akhter S, Islam MS, Dash AP, Kroeger A, Banu Q. Indoor residual spraying for kala-azar vector control in Bangladesh: A continuing challenge. *PLoS Negl Trop Dis.* 2018 Oct 1;12(10):e0006846.
154. Su T, Thieme J, Cummings R, Cheng ML, Brown MQ. Cross Resistance in S-Methoprene-Resistant *Culex quinquefasciatus* (Diptera: Culicidae). *J Med Entomol.* 2021 Jan 12;58(1):398-402.
155. Dormont L, Mulatier M, Carrasco D, Cohuet A. Mosquito Attractants. *J Chem Ecol.* 2021 May;47(4-5):351-93.
156. Pavela R, Maggi F, Iannarelli R, Benelli G. Plant extracts for developing mosquito larvicides: From laboratory to the field, with insights on the modes of action. *Acta Trop.* 2019 May;193:236-71.
157. Mali RG. *Cleome viscosa* (wild mustard): a review on ethnobotany, Phytochemistry, and Pharmacology. *Pharm Biol.* 2010 Jan;48(1):105-12.
158. Krishnappa K, Elumalai K. Mosquitocidal properties of *Basella rubra* and *Cleome viscosa* against *Aedes aegypti* (Linn.) (Diptera: Culicidae). *Eur Rev Med Pharmacol Sci.* 2013 May;17(9):1273-77.
159. Phowichit S, Buatippawan S, Bullangpoti V. Insecticidal activity of *Jatropha gossypifolia* L. (Euphorbiaceae) and *Cleome viscosa* L. (Capparidaceae) on *Spodoptera litura* (Lepidoptera: Noctuidae). Toxicity and carboxylesterase and glutathione-S-transferase activities studies. *Commun Agric Appl Biol Sci.* 2008;73(3):611-9.

160. Bhattacharya E; Dutta R, Chakraborty S, Mandal BS. Phytochemical profiling of *Artocarpus lakoocha* Roxb. leaf methanol extract and its antioxidant, antimicrobial and antioxidative activities. *Asian Pacific J of Tropical Biomedicine*. 2019; 9(11):484-92.
161. Gupta AK, Rather MA, Kumar Jha A, Shashank A, Singhal S, Sharma M, Pathak U, Sharma D, Mastinu A. *Artocarpus lakoocha* Roxb. and *Artocarpus heterophyllus* Lam. Flowers: New Sources of Bioactive Compounds. *Plants (Basel)*. 2020 Oct 9;9(10):1329.
162. Preyavichyapugdee N, Sangfuang M, Chaiyapum S, Sriburin S, Pootaeng-on Y, Chusongsang P, Jiraungkoorskul W, Preyavichyapugdee M, Sobhon P. Schistosomicidal activity of the crude extract of *Artocarpus lakoocha*. *Southeast Asian J Trop Med Public Health*. 2016 Jan;47(1):1-15.
163. Saowakon N, Tansatit T, Wanichanon C, Chanakul W, Reutrakul V, Sobhon P. *Fasciola gigantica*: anthelmintic effect of the aqueous extract of *Artocarpus lakoocha*. *Exp Parasitol*. 2009 Aug;122(4):289-98.
164. Revathy SS, Rathinamala R, Murugesan M: Authentication Methods For Drugs Used In Ayurveda, Siddha And Unani Systems Of Medicine: An Overview. *Int J Pharm Sci Res*, 2012; Vol. 3(8): 2352-61.
165. Guidelines for assessing the quality of herbal medicines with reference to contaminants and residues. World Health Organization, 2007.
166. Bijauliya RK, Alok S, Chanchal DK and Kumar M: A comprehensive review on standardization of herbal drugs. *Int J Pharm Sci Res* 2017;8(9):3663-77.
167. Sachan, Anupam & Vishnoi, Garima & Kumar, Roopak. Need of standardization of herbal medicines in the Modern era. *Intern. J of Phytomedicine*. 2016;8:300.
168. Bruckner, James V., et al. Chapter 24. Toxic Effects of Solvents and Vapors. *Casarett & Doull's Essentials of Toxicology*, 2nd Ed. Curtis D. Klaassen, and John B. Watkins, III. McGraw Hill, 2010, <https://accesspharmacy.mhmedical.com/content.aspx?bookid=449§ionid=39910793>.
169. Prabh SS, Mandeep B, Hayat MM, Gagan S. Standardization and pharmacological investigation on leaves of *Ficus bengalensis*. *IJRPC* 2011,1(4):891-903

- 170.Kadam VR. Determination of Ash Values of Some Medicinal Plants of Genus *Sesbania* of Marathwada Region in Maharashtra. *Current Drug Discovery Technol.* 2011;1:193-195.
- 171.Alam F, Us Saqib QN. Pharmacognostic study and development of quality control parameters for fruit, bark and leaf of *Zanthoxylum armatum* (Rutaceae). *Anc Sci Life.* 2015 Jan-Mar;34(3):147-55.
- 172.Akbar S, Hanif U, Ali J, Ishtiaq S. Pharmacognostic studies of stem, roots and leaves of *Malva parviflora* L. *Asian Pac J Trop Biomed.* 2014 May;4(5):410-15.
- 173.Pandey A, Bhatnagar SP. Preliminary Phytochemical screening and antimicrobial studies on *Artocarpus lakoocha* Roxb. *Anc Sci Life.* 2009 Apr;28(4):21-4.
- 174.Pillai LS, Nair BR. A comparative study of the anthelmintic potential of *Cleome viscosa* L. And *Cleome burmanni* w. And a. *Indian J Pharm Sci.* 2011 Jan;73(1):98-100.
- 175.Devi BP, Boominathan R, Subhash, Mandal C. Studies of pharmacognostical profiles of *Cleome viscosa* L (family:capparidaceae). *Anc Sci Life.* 2003 Apr;22(4):115-21.
- 176.Krishnaiah D, Nithyanandam R, Sarbatly R. A critical review on the spray drying of fruit extract: effect of additives on physicochemical properties. *Crit Rev Food Sci Nutr.* 2014;54(4):449-73.
- 177.Gallo L, Bucalá V. A Review on Influence of Spray Drying Process Parameters on the Production of Medicinal Plant Powders. *Curr Drug Discov Technol.* 2019;16(4):340-354.
- 178.Geneva: World Health Organization; 2017 (WHO/HTM/NTD/WHOPES/2017.04). Licence: CC BY-NC-SA 3.0 IGO.
- 179.Sakthivadivel M, Gunasekaran P, Mathew J, Samraj A, Arivoli S, Tennyson S. Evaluation of larvicidal efficacy of *Cleome viscosa* L. (Capparaceae) aerial extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae), *Asian Pacific J of Tropical Dis.* 2014;4(2):S795-S798.
- 180.Islam MM, Islam MZ, Shaekh MPE, Das P, ChowdhuryHK, Shahik SM, Muzahid NH, Khan MA, Ekram AE. Screening of *Cleome viscosa* (L.) for Dose Mortality, Insect Repellency, Cytotoxicity and Larvicidal Activities in the Laboratory Condition. *Intern. J of Scientific & Engineering Res.,* 2014;5(1):2201-15.

181. Pineda-Cortel MRB, Cabantog RJR, Caasi PM, Ching CAD, Perez JBS, Godisan PGM, et al. Larvicidal and ovicidal activities of *Artocarpus blancoi* extracts against *Aedes aegypti*. *Pharm Biol.* 2019 Dec;57(1):120-24.
182. Day JF. Mosquito Oviposition Behavior and Vector Control. *Insects.* 2016 Nov 18;7(4):65.
183. Upadhyay RK, Yadav N. Insecticidal potential of aqueous and solvent extracts of *Cassia fistula*, *Cleome viscosa* and *Capparis decidua* against *Callosobruchus chinensis* L. (Coleoptera: Bruchidae). *Intern. J of Chemical and Biochemical Sciences.* 2012;1:91-8.
184. Chinnamma CA. Influence of Petroleum Ether Leaf Extract of *Vitex Negundo* on the Oviposition Deterrence of Adult Uzi fly (*Exorista bombycis*). *Intern. J of Innovative Sci. and Res. Technol.* 2017;2(5):31-7.
185. Ali SI, Venkatesalu V. Evaluation of the larvicidal potential of root and leaf extracts of *Saussurea costus* (Falc.) Lipsch. against three mosquito vectors: *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*. *Rev Soc Bras Med Trop.* 2020 Mar 16;53:e20190018.
186. Xu P, Zeng F, Bedoukian RH, Leal WS. DEET and other repellents are inhibitors of mosquito odorant receptors for oviposition attractants. *Insect Biochem Mol Biol.* 2019 Oct;113:103224.
187. Xue RD, Ali A, Crainich V, Barnard D. Oviposition deterrence and larvicidal activity of formulations of piperidine repellent (AI3-37220) against field populations of *Stegomyia albopicta*. *J Am Mosq Control Assoc.* 2007 Sep;23(3):283-7.
188. Rajkumar S, Jebanesan A. Oviposition deterrent and skin repellent activities of *Solanum trilobatum* leaf extract against the malarial vector *Anopheles stephensi*. *J Insect Sci.* 2005;5:15.
189. Swathi S, Murugananthan G, Ghosh SK. Oviposition deterrent activity from the ethanolic extract of *Pongamia pinnata*, *Coleus forskohlii*, and *Datura stramonium* leaves against *Aedes aegypti* and *Culex quinquefasciatus*. *Pharmacogn Mag.* 2010 Oct;6(24):320-2.
190. Reegan AD, Gandhi MR, Paulraj MG, Ignacimuthu S. Ovicidal and Oviposition Deterrent Activities of Medicinal Plant Extracts against *Aedes aegypti* L. and *Culex quinquefasciatus* Say Mosquitoes (Diptera: Culicidae). *Osong Public Health Res Perspect.* 2015 Feb;6(1):64-9

191. Prajapati V, Tripathi AK, Aggarwal KK, Khanuja SP. Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Bioresour Technol.* 2005 Nov;96(16):1749-57.
192. Martianasari R. Larvicidal, adulticidal, & oviposition-deterrent activity of Piper betle L. essential oil to *Aedes aegypti*. *Vet World.* 2019;12(3):367-371.
193. Nobsathian S, Bullangpoti V, Kumrungsee N, Wongsan N, Ruttanakum D. Larvicidal effect of compounds isolated from *Maerua siamensis* against *Aedes aegypti* (Diptera: Culicidae) larvae. *Chem. Biol. Technol. Agric.* 2018; 5:8.
194. Suter T et al. Insecticide susceptibility of *Aedes albopictus* and *Ae. aegypti* from Brazil and the Swiss-Italian border region. *Parasit Vectors.* 2017 Sep 19;10(1):431.
195. Rahuman AA, Gopalakrishnan G, Venkatesan P, Geetha K, Bagavan A. Mosquito larvicidal activity of isolated compounds from the rhizome of *Zingiber officinale*. *Phytother Res.* 2008 Aug;22(8):1035-9.
196. Khanavi M, Najafi B, Sadati SN, Abai MR, Vatandoost H. Chemical Constituents and Larvicidal Activity of Fractions of *Ajuga chamaecistus tomentella* against Malaria Vector *Anopheles stephensi*. *J Arthropod Borne Dis.* 2017 Mar 14;11(1):116-123.
197. Deore S, Khadabadi S. Larvicidal activity of the saponin fractions of *Chlorophytum borivilianum* Santapau and Fernandes. *J of Entomology and Nematology.* 2009;1(5):064-66.
198. PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 5318645, isorhamnetin 3-O-glucoside; [cited 2024 Mar. 9].
199. PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 10006384, Quercetin 3-O-(6"-acetyl-glucoside); [cited 2024 Mar.9].
200. PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 6911854, Sinigrin;
201. PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 3806, Juglone;

202. PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 92110, Cycloartenol;
203. National Center for Biotechnology Information (2020). PubChem Compound Summary for CID 3012524, lakoochin A.
204. National Center for Biotechnology Information (2020). PubChem Compound Summary for CID 14237687, Albafuran B.
205. Almeida TC, Larentis AL, Ferraz HC. Evaluation of the stability of concentrated emulsions for lemon beverages using sequential experimental designs. PLoS One. 2015 Mar 20;10(3):e0118690.
206. Yi B, Kim MJ, Lee J. Antioxidant Properties of Astaxanthin in Oil-in-Water Emulsions with Differently-Charged Emulsifiers Under Chlorophyll Photosensitization. J Food Sci. 2018 Mar;83(3):589-96.
207. Dhra G, Nusrat, Bhandari P, Kumar J, Patanjali PK (2017) Advanced Pesticide Formulations for Dengue Preventions. J Fever. 2017;1(2):1007-12.
208. Jean-Louis Salager (2000). Emulsion Properties and related know-how to attain them. In: Pharmaceutical Emulsions and Suspensions. Francoise Nielloud and Gilberte Marti-Mestres (ED), Marcel, dekker, INC. 76-92 p.
209. McClements, D. Emulsions: principles, practice and techniques. Boca Raton, CRC Press. 2005; 456-96 p.
210. Ye A, Hemar Y, Singh H. Enhancement of coalescence by xanthan addition to oil-in-water emulsions formed with extensively hydrolysed whey proteins. Food Hydrocolloids. 2004;18(5):737-46.
211. Lawlor JA, Arellano SM. Temperature and salinity, not acidification, predict near-future larval growth and larval habitat suitability of Olympia oysters in the Salish Sea. Sci Rep. 2020 Aug 14;10(1):13787.
212. Robert L. Zimdahl, Chapter 17 - Herbicide Formulation, Editor(s): Robert L. Zimdahl, Fundamentals of Weed Sci. (Fifth Edition), Academic Press, 2018, 501-509.
213. Reißner AM, Al-Hamimi S, Quiles A, Schmidt C, Struck S, Hernando I, Turner C, Rohm H. Composition and physicochemical properties of dried berry pomace. J Sci Food Agric. 2019 Feb;99(3):1284-1293.
214. Jamnback H, Frempong-Boadu J. Testing blackfly larvicides in the laboratory and in streams. Bull World Health Organ. 1966;34(3):405-21.

215. Haq S, Bhatt RM, Vaishnav KG, Yadav RS. Field evaluation of bio-larvicides in Surat city, India. J Vector Borne Dis. 2004 Sep-Dec;41(3-4):61-6.
216. Leon Lachman. Physical and Chemical Stability Testing of Tablet Dosage Forms, J of Pharmaceutical Sci. 1965;54(10):1519-26.

ANNEXURE

ANNEXURE I: Plant Authentication Certificates



25 December 2014

Authentication certificate

Name of the species : *Cleome viscosa* L.

Synonym : *Arivela viscosa* (L.) Raf., *Cleome acutifolia* Elmer, *Polanisia icosandra* (L.) Wight & Arn., *Polanisia viscosa* (L.) Blume, *Sinapisrum viscosum* (L.) Moench

Part : Whole plant

Family : Cleomaceae

Common name : Naibela

Batch No : 221/2014

Place of Collection : Western Ghats

Date : 25th Aug 2014

Characteristic features: Erect herb growing open land and rainfed cultivation. Herb covered with glandular trichome, leaves alternate, petiole, long, leaves, 3- 5foliate, leaflets round or obovate, margin entire, flowers from the upper leaf angles, petals yellowish, fruit long capsule.

Identity confirmed by: Dr. P. Santhan
Plant Taxonomist

Dr. P. SANTHAN
Plant Taxonomist

Regd. Office & R & D

Plot No. 5B, Veerasandra Indl. Area, 19th K.M. Stone, Hosur Road, Electronic City Post, Bangalore - 560 100, INDIA
Tel : +91-80-4020 9999, 2783 2265, Fax : +91-80-4020 9817, CIN No. : U24232KA1998PTC023573
E-mail : research@naturalremedy.com, Web : www.naturalremedy.com

Natural Solutions for Healthy Living

25 December 2014

Authentication certificate

Name of the species : *Artocarpus lacucha* Buch.-Ham.

Synonym : *Artocarpus lakoocha* Roxb.,

Part : Leaves

Family : Moraceae

Common name : Monkey jack

Batch No : 223/2014

Place of Collection : Western Ghats

Date : 29 April 2014

Characteristic features: Leaves alternate, 10-25 cm long, elliptic, pointed and leathery, pubescent abaxially. Flowers unisexual-male and female flowers in separate spherical heads, but on the same tree. Male flowers are yellow-orange while the female are reddish. Fruits a syncarp (the entire female inflorescence forms a fruit), regular round, green when young, turning yellow at the time of maturity, later brown. The size differs but the diameter is typically 5-10 cm while fruit weights 200-350 g. The number of seeds/fruit varies accordingly, but typically there are 10-30 per fruit. Seeds irregular and vary in size like the fruits. Fruit rind is acid to taste and used in cooking.

Identity confirmed by: Dr. P. Santhan
Plant Taxonomist

Dr. P. SANTHAN
Plant Taxonomist

Regd. Office & R & D

Plot No. 5B, Veerasandra Indl. Area, 19th K.M. Stone, Hosur Road, Electronic City Post, Bangalore - 560 100, INDIA
Tel : +91-80-4020 9999, 2783 2265, Fax : +91-80-4020 9817, CIN No. : U24232KA1998PTC023573
E-mail : research@naturalremedy.com, Web : www.naturalremedy.com

Natural Solutions for Healthy Living

25 December 2014

Authentication certificate

Name of the species : *Artocarpus lacucha* Buch.-Ham.

Synonym : *Artocarpus lakoocha* Roxb.,

Part : Fruit

Family : Moraceae

Common name : Monkey jack

Batch No : 224/2014

Place of Collection : Western Ghats

Date : 29 April 2014

Characteristic features: Leaves alternate, 10-25 cm long, elliptic, pointed and leathery, pubescent abaxially. Flowers unisexual-male and female flowers in separate spherical heads, but on the same tree. Male flowers are yellow-orange while the female are reddish. Fruits a syncarp (the entire female inflorescence forms a fruit), regular round, green when young, turning yellow at the time of maturity, later brown. The size differs but the diameter is typically 5-10 cm while fruit weights 200-350 g. The number of seeds/fruit varies accordingly, but typically there are 10-30 per fruit. Seeds irregular and vary in size like the fruits. Fruit rind is acid to taste and used in cooking.

Identity confirmed by: Dr. P. Santhan
Plant Taxonomist



Dr. P. SANTHAN
Plant Taxonomist

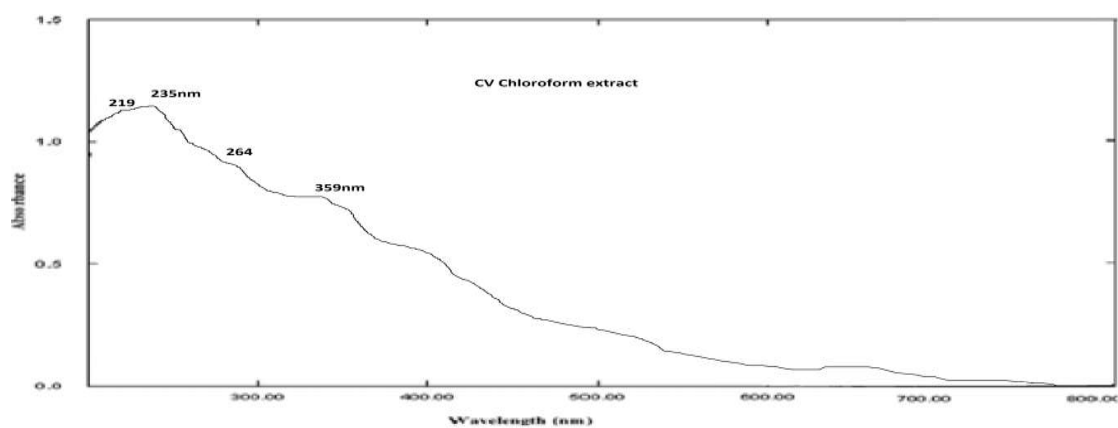
Regd. Office & R & D

Plot No. 5B, Veerasandra Indl. Area, 19th K.M. Stone, Hosur Road, Electronic City Post, Bangalore - 560 100, INDIA
Tel : +91-80-4020 9999, 2783 2265, Fax : +91-80-4020 9817, CIN No. : U24232KA1998PTC023573
E-mail : research@naturalremedy.com, Web : www.naturalremedy.com

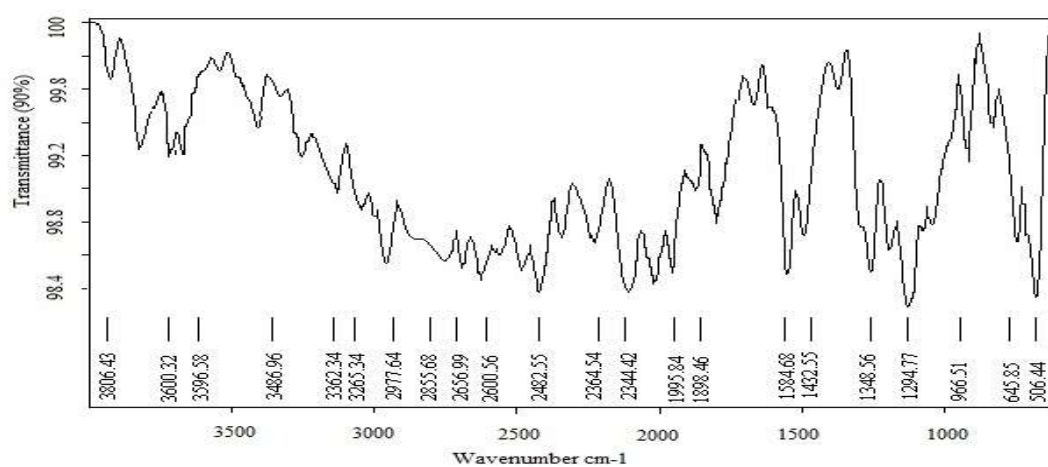
Natural Solutions for Healthy Living

9.2: ANNEXURES II: SPECTROSCOPICAL DATA

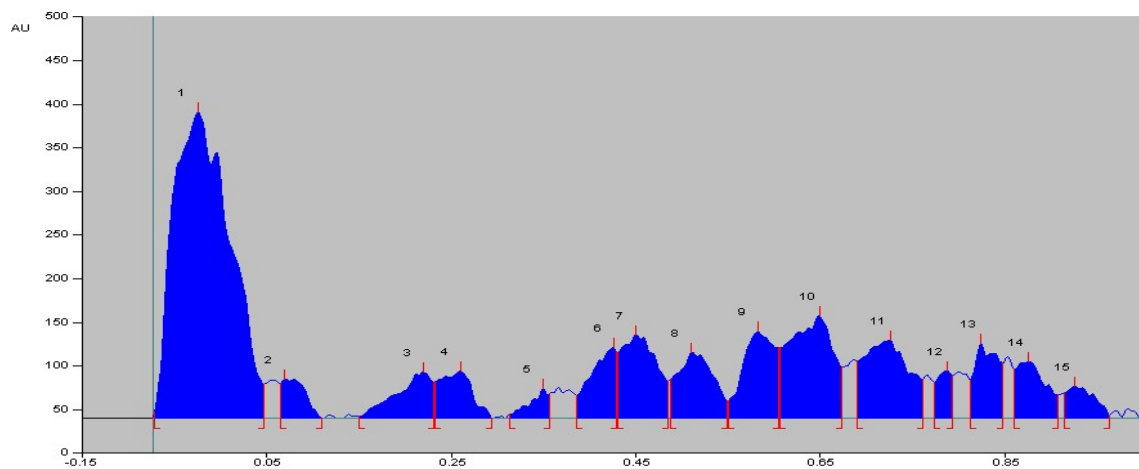
9.2.1: UV absorption spectra of CHE of CVA



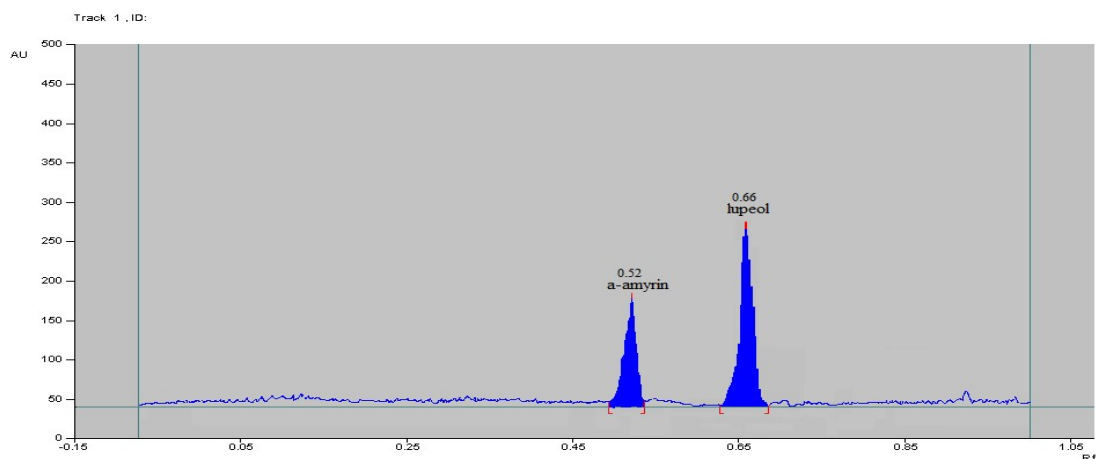
9.2.2: IR spectra of CHE of CVA



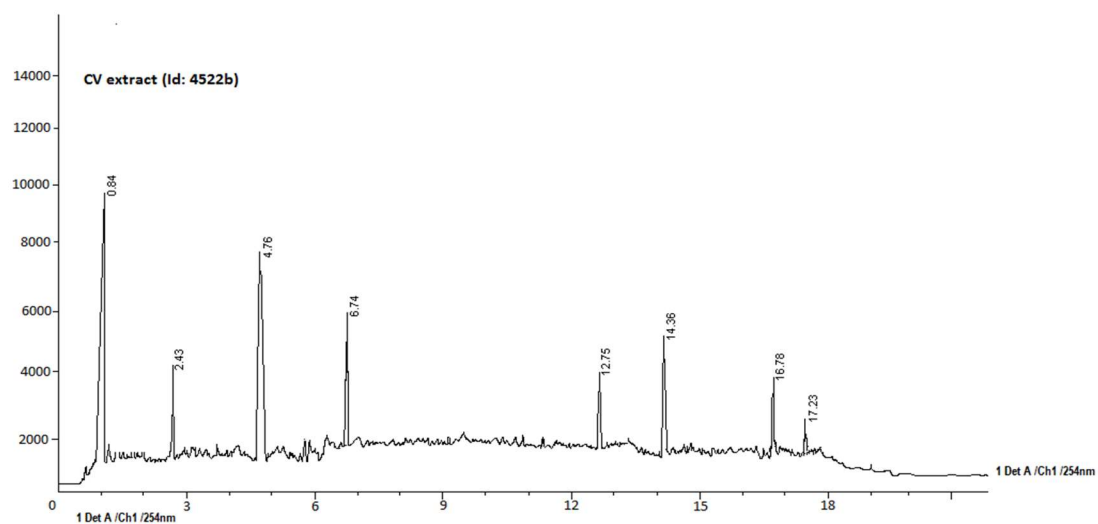
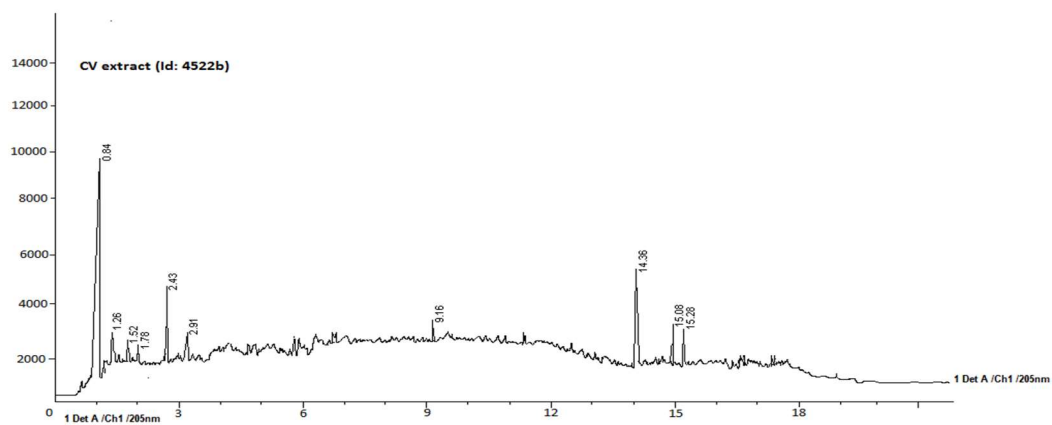
9.2.3: HPTLC fingerprint of CHE of CVA

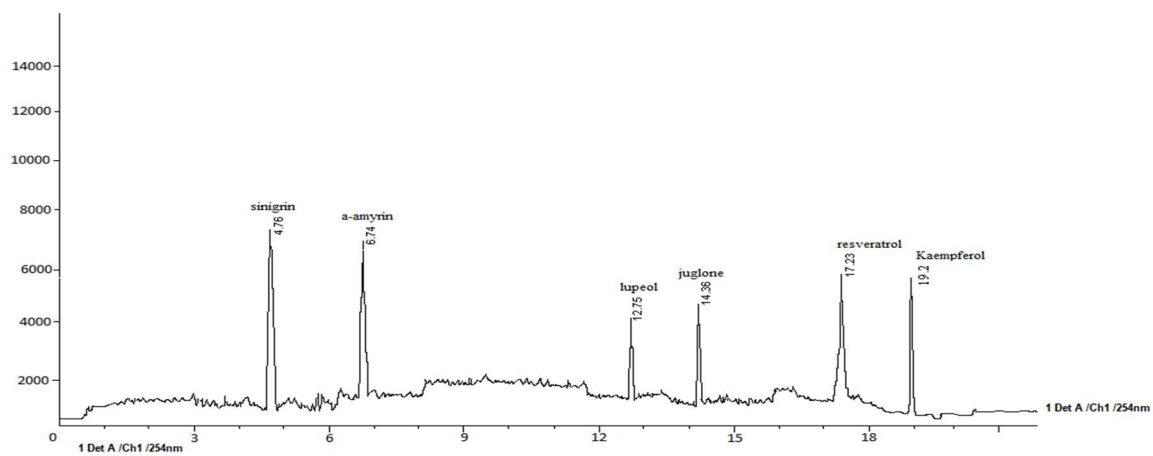
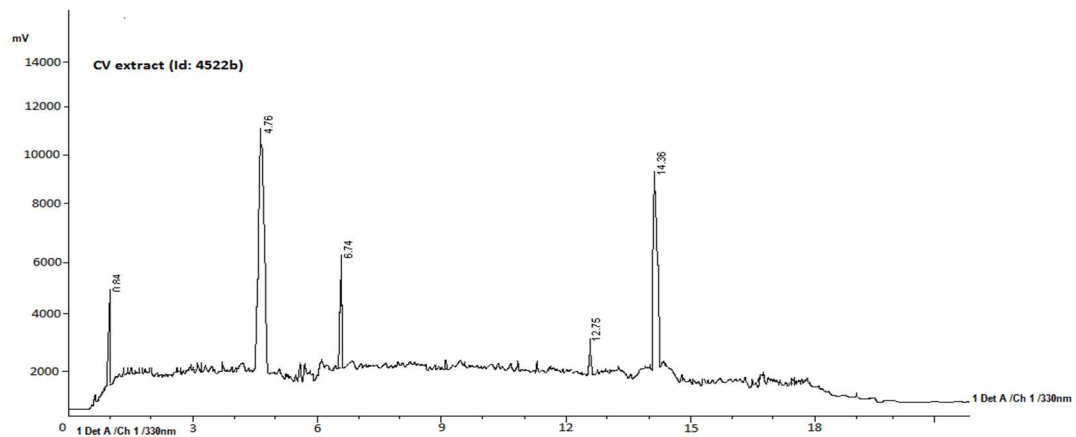


9.2.3.1: HPTLC fingerprint of standards α - amyrin and lupeol



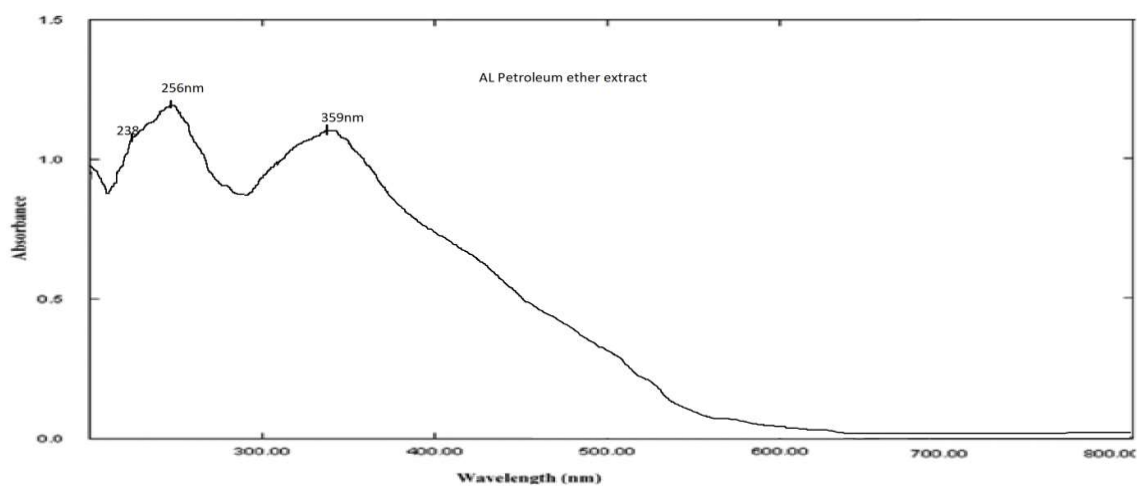
9.2.4: HPLC of CHE of CVA



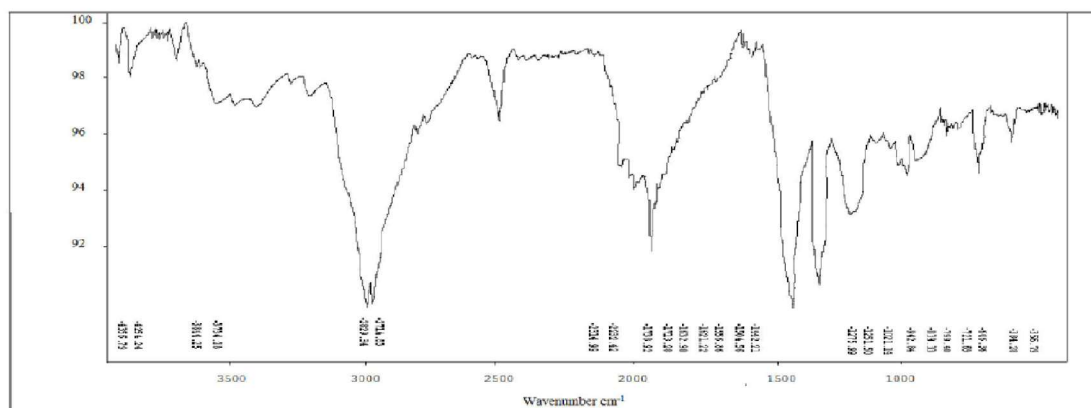


Standards sinigrin, α -amyrin, lupeol, juglone, resveratrol & kaempferol at 254nm

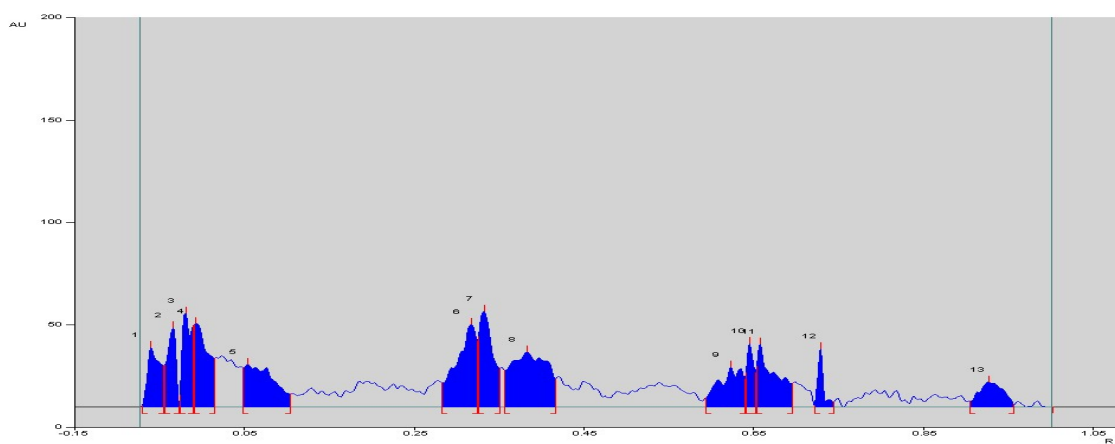
9.2.5: UV spectra of PEE of ALL



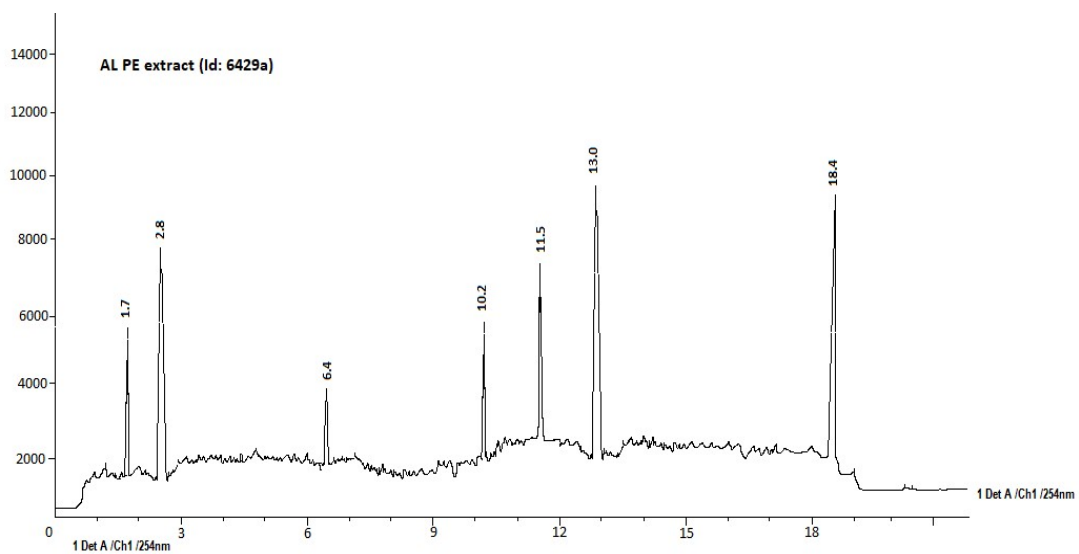
9.2.6: IR spectra of PEE of ALL

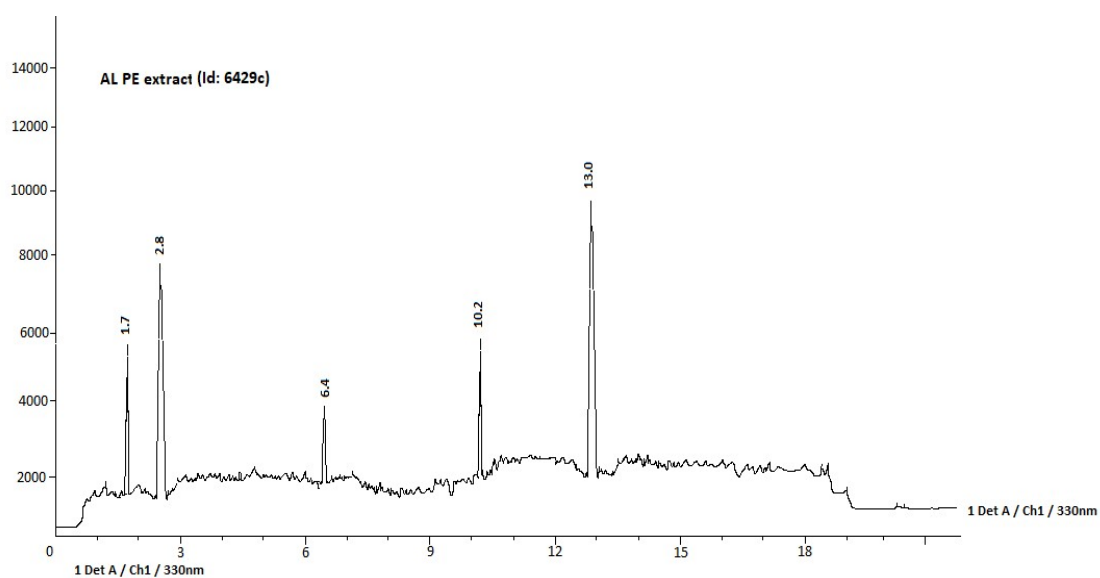
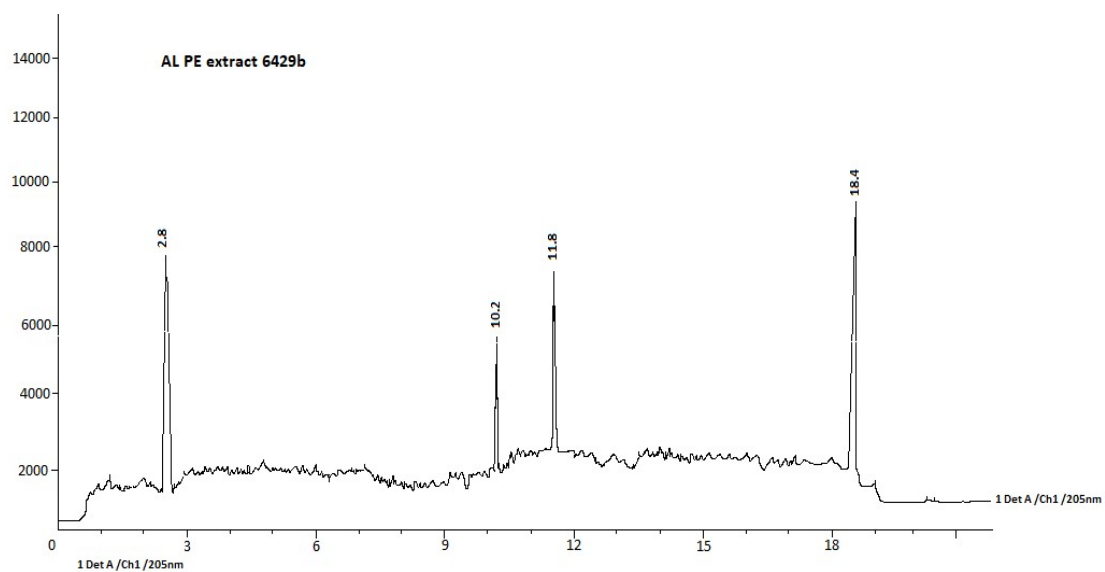


9.2.7: HPTLC fingerprint of PEE of ALL

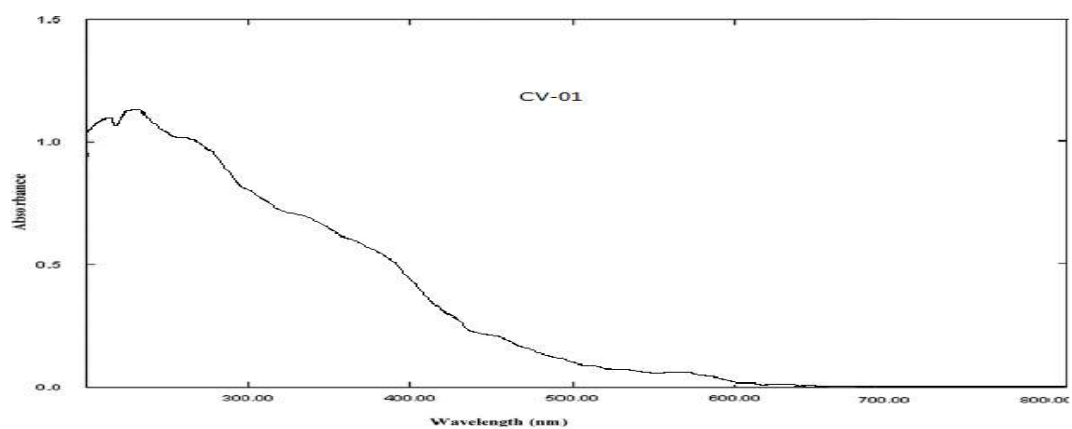


9.2.8: HPLC of PEE of ALL

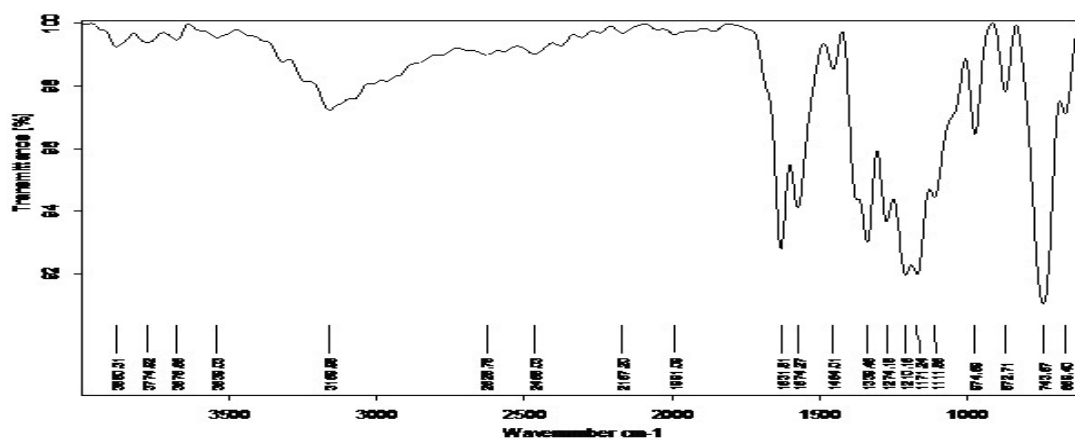




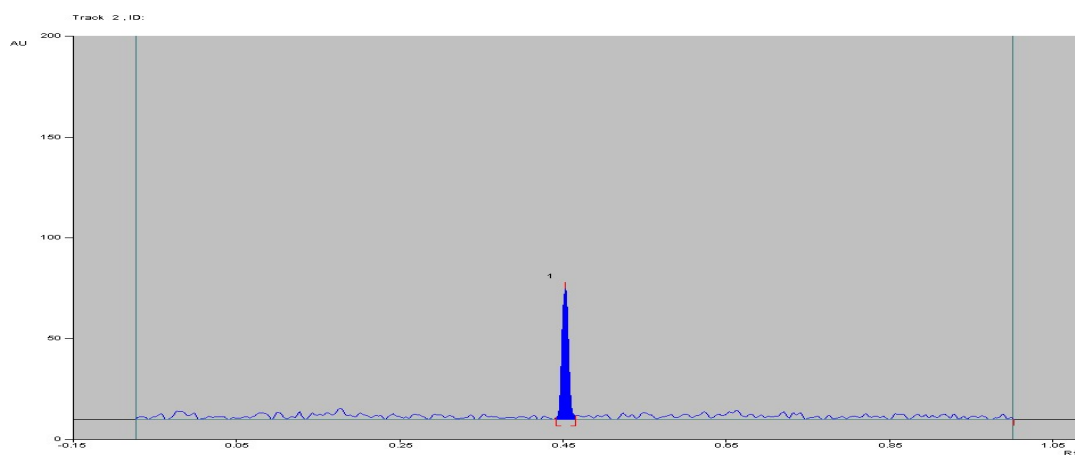
9.2.9: UV spectra of isolated Compound CV/C1/F6-8/C2/F4/comp-03



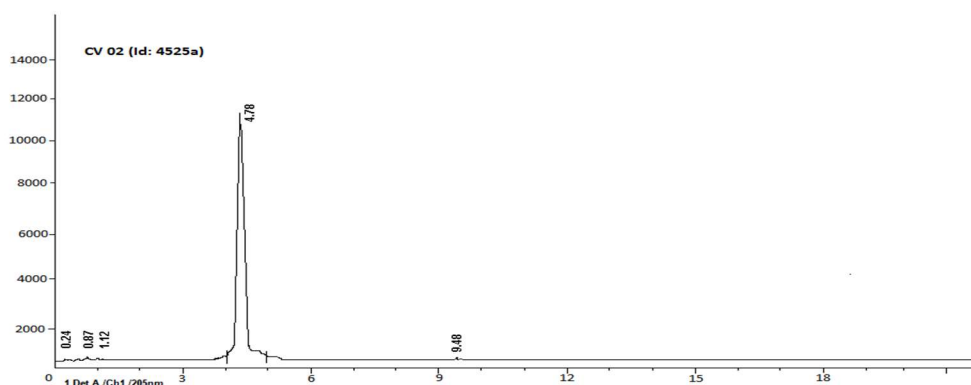
9.2.10: IR spectra of isolated Compound CV/C1/F6-8/C2/F4/comp-03

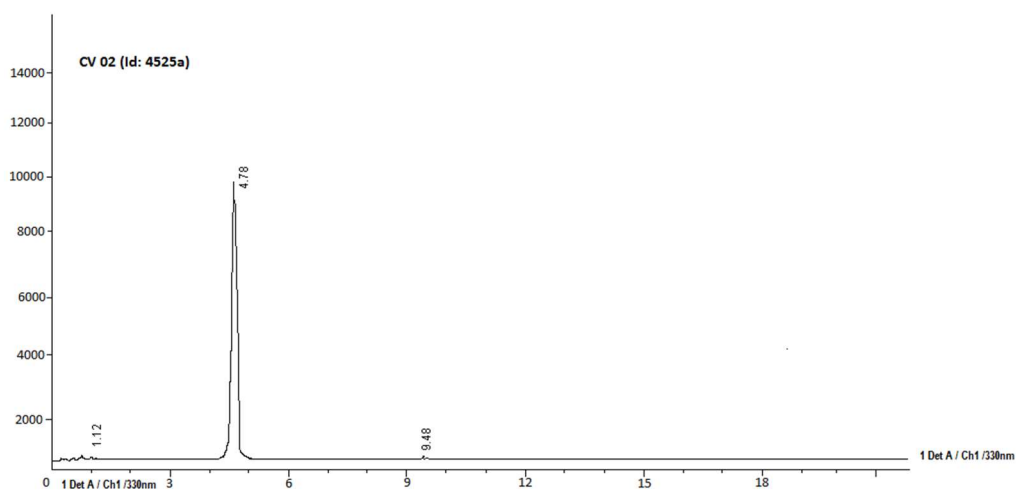
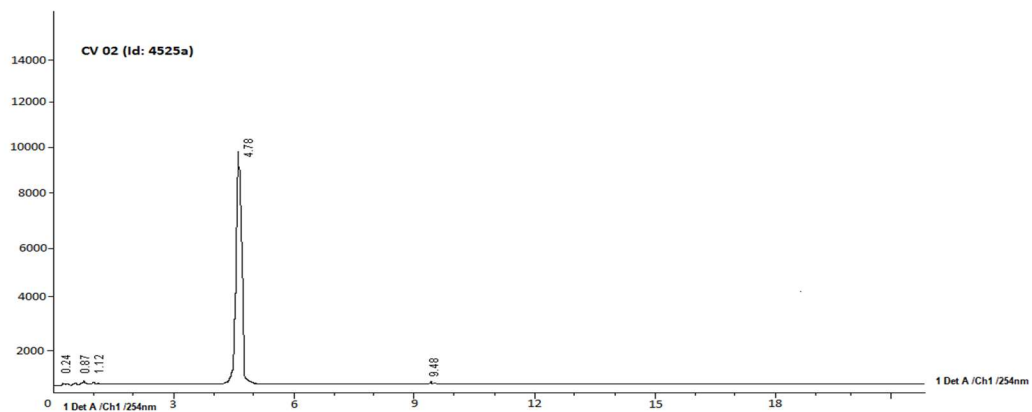


9.2.11: HPTLC of Isolated compound CV/C1/F6-8/C2/F4/comp-03

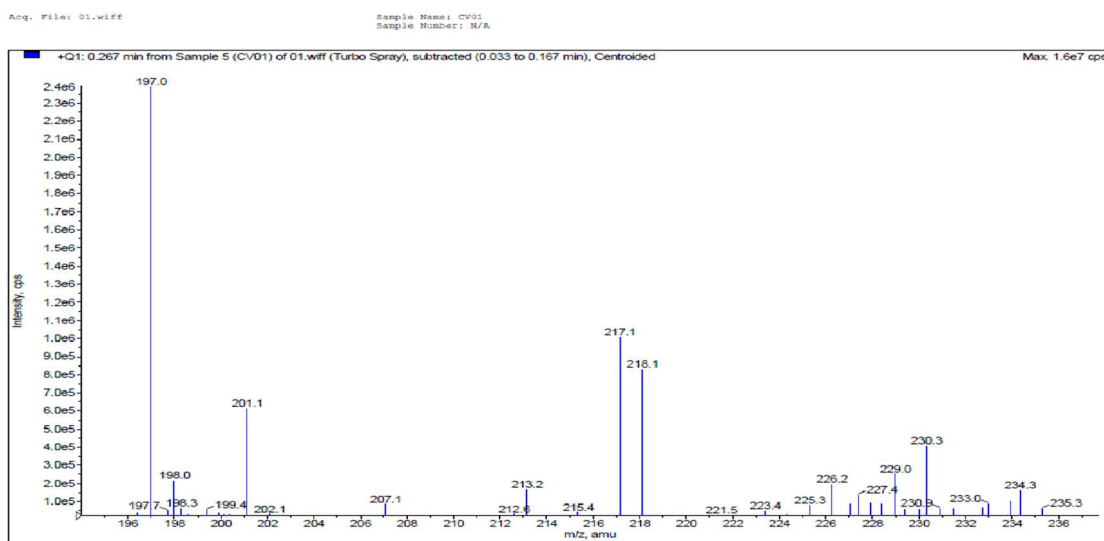


9.2.12: HPLC of Isolated compound CV/C1/F6-8/C2/F4/comp-03

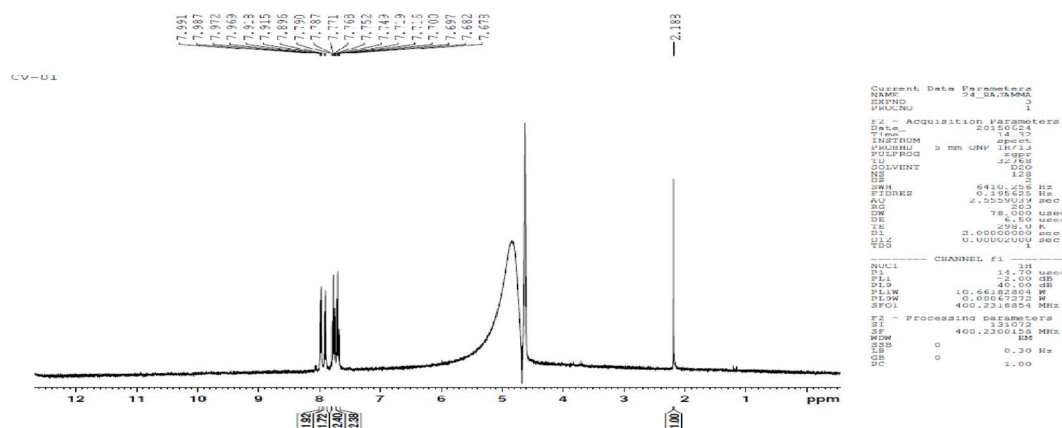




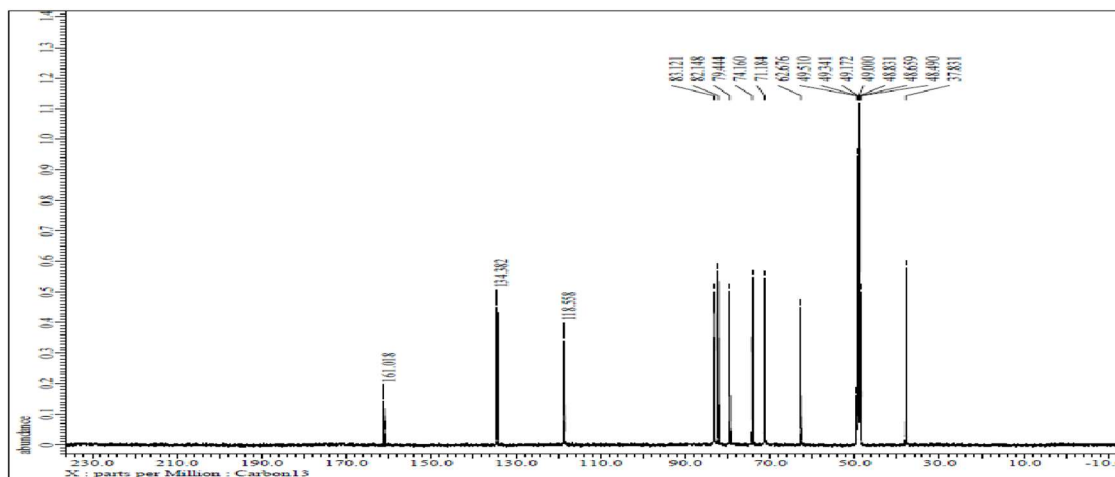
9.2.13: Mass spectra of isolated Compound CV/C1/F6-8/C2/F4/comp-03



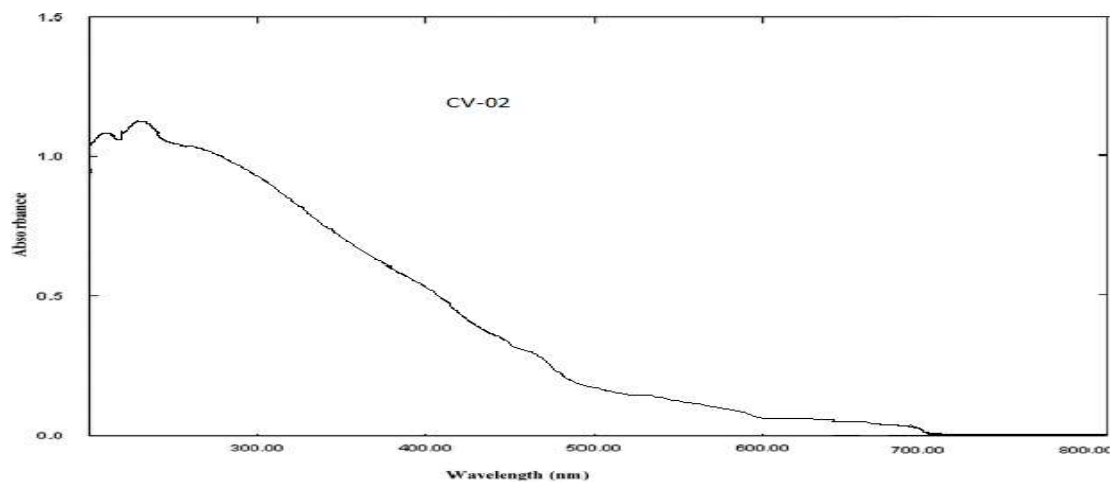
9.2.14: ^1H NMR of isolated Compound CV/C1/F6-8/C2/F4/comp-03



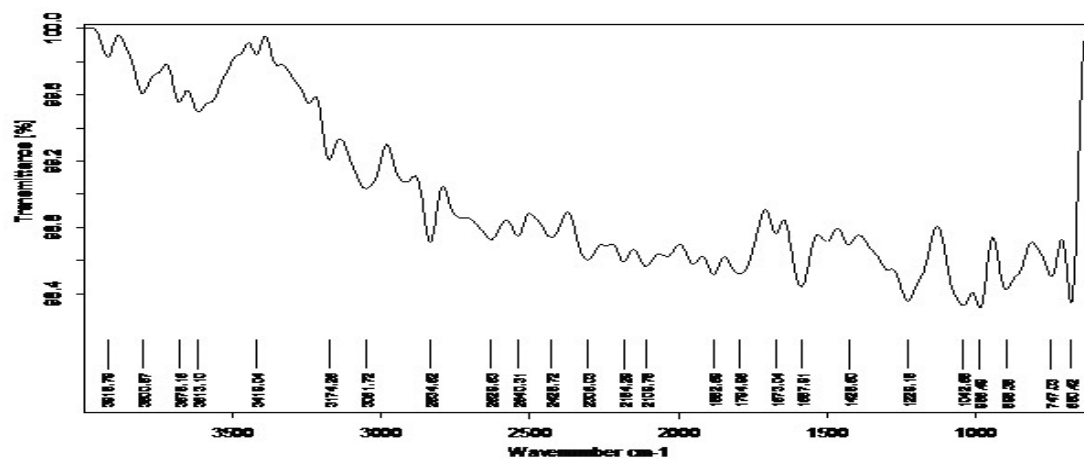
9.2.15: ^{13}C NMR of isolated Compound CV/C1/F6-8/C2/F4/comp-03



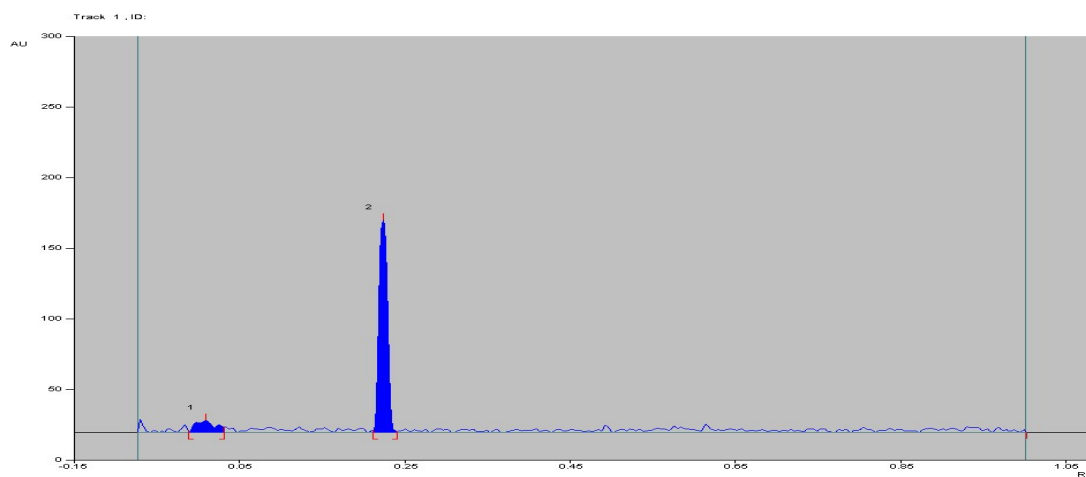
9.2.16: UV absorption spectra of isolated Compound CV/C1/F16-17/04



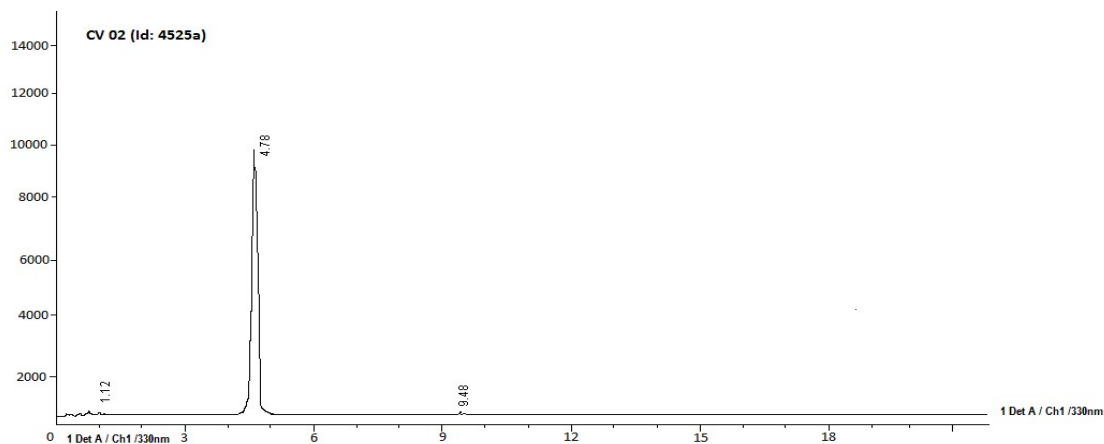
9.2.17: IR spectra of isolated Compound CV/C1/F16-17/04

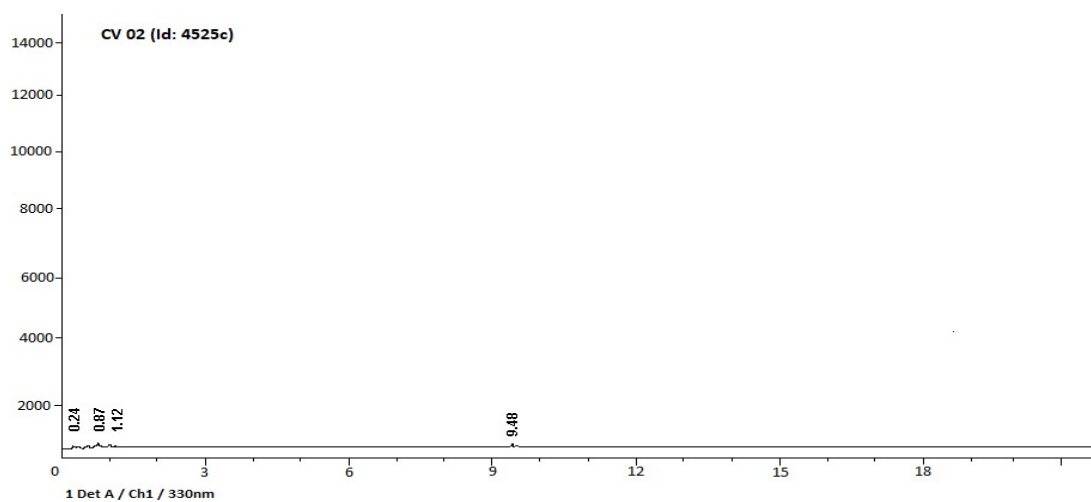
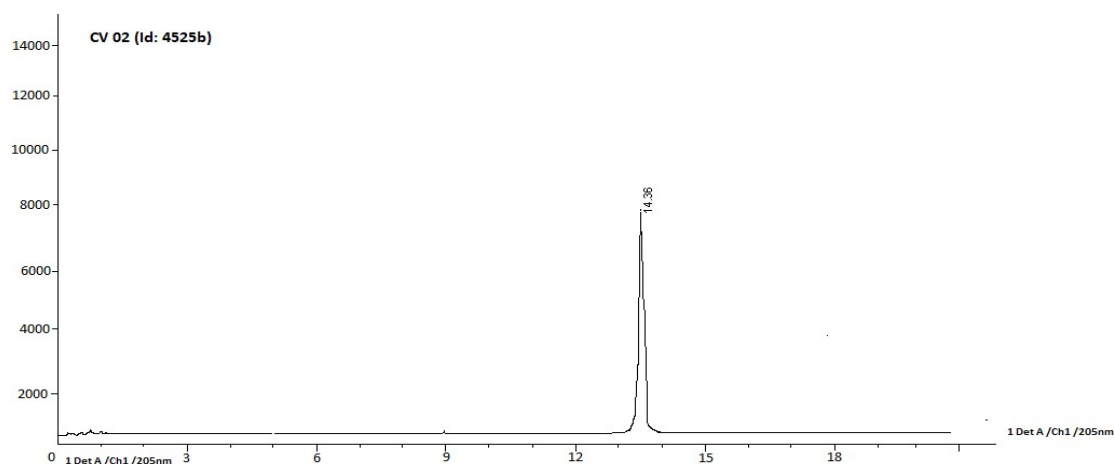


9.2.18: HPTLC spectra of isolated Compound CV/C1/F16-17/04

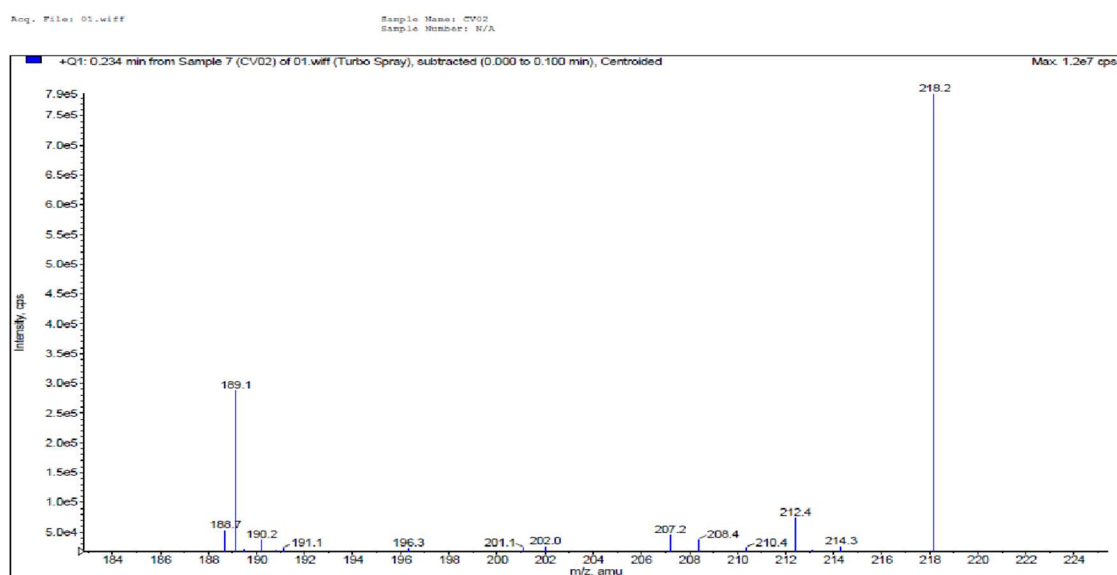


9.2.19: HPLC of isolated Compound CV/C1/F16-17/04

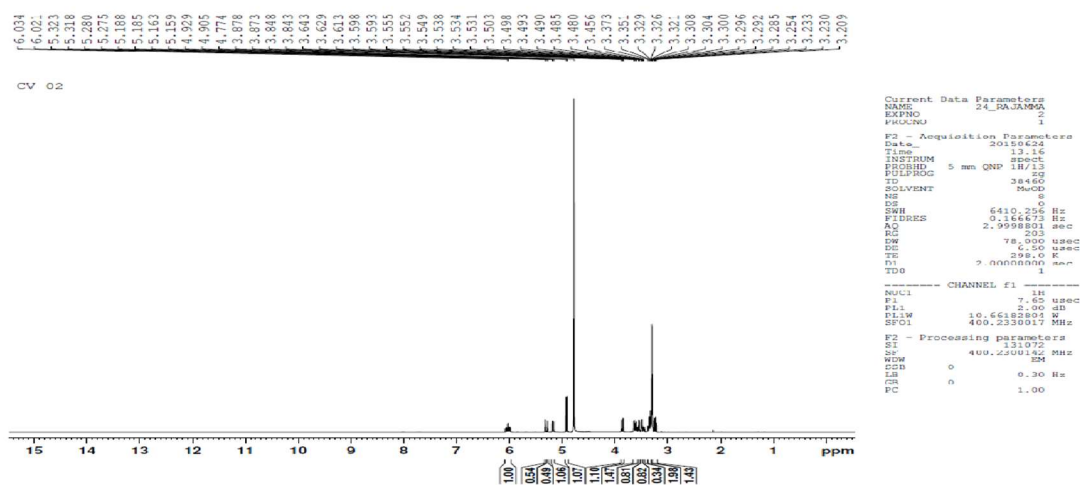




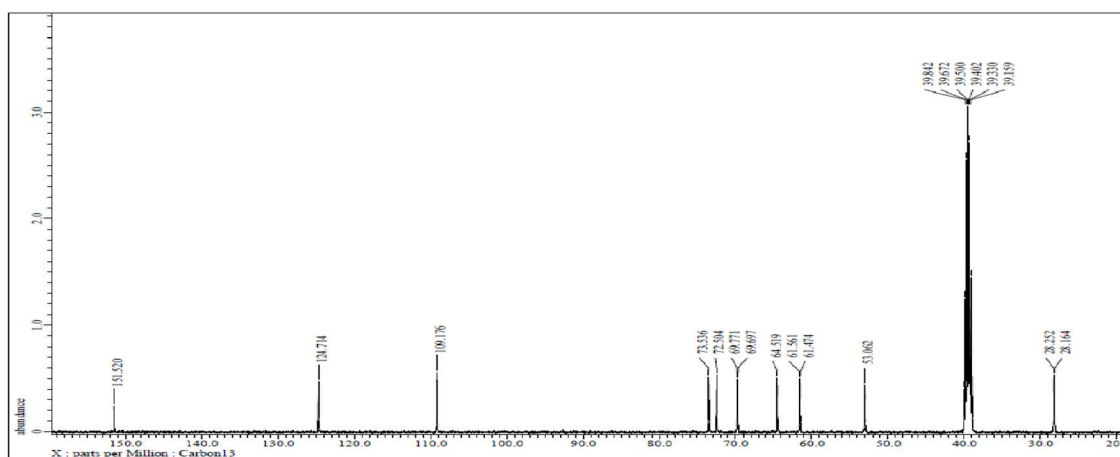
9.2.20: Mass spectra of isolated Compound CV/C1/F16-17/04



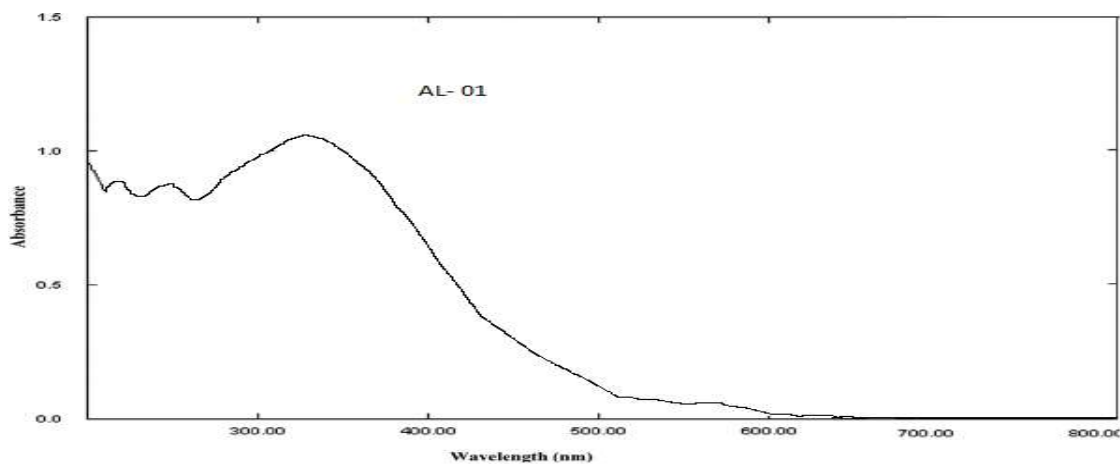
9.2.21: ^1H NMR spectra of isolated Compound CV/C1/F16-17/04



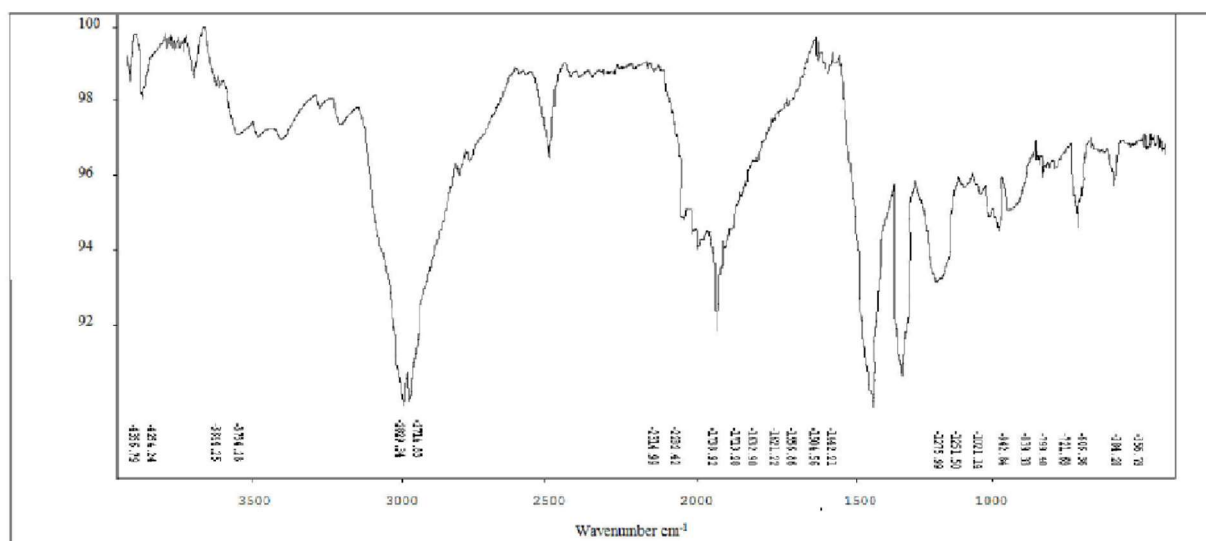
9.2.22: ^{13}C NMR spectra of isolated Compound CV/C1/F16-17/04



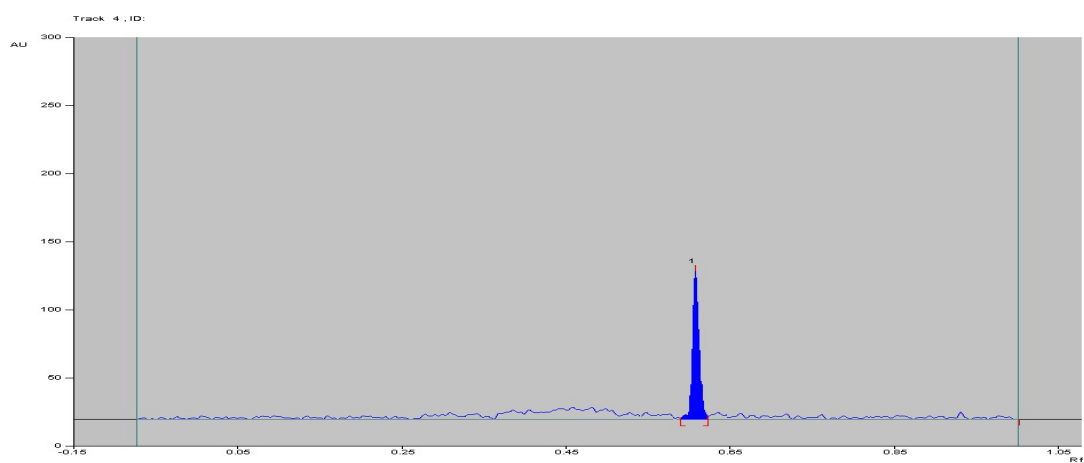
9.2.23: UV spectra of isolated Compound ALL/C1/F4-5/C2/F1-2/comp-01



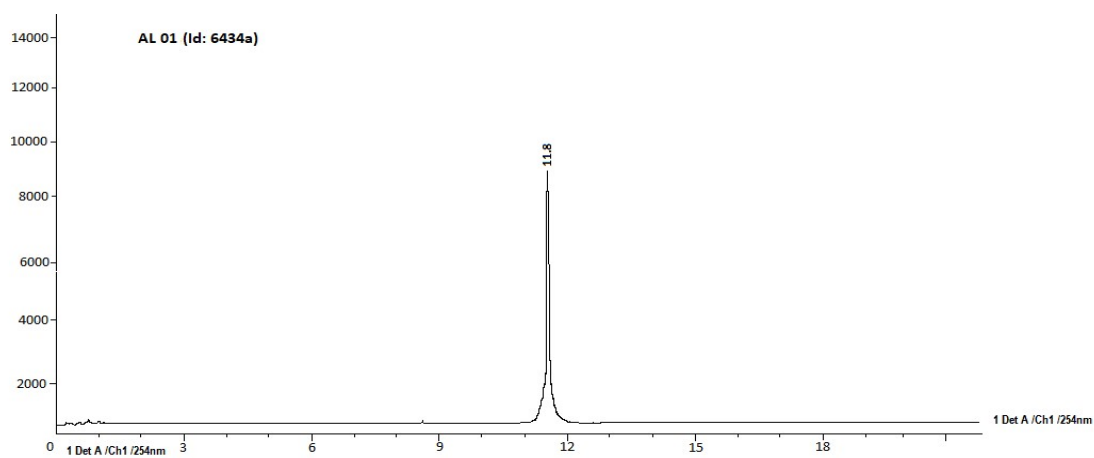
9.2.24: IR spectrum of isolated Compound ALL/C1/F4-5/C2/F1-2/comp-01



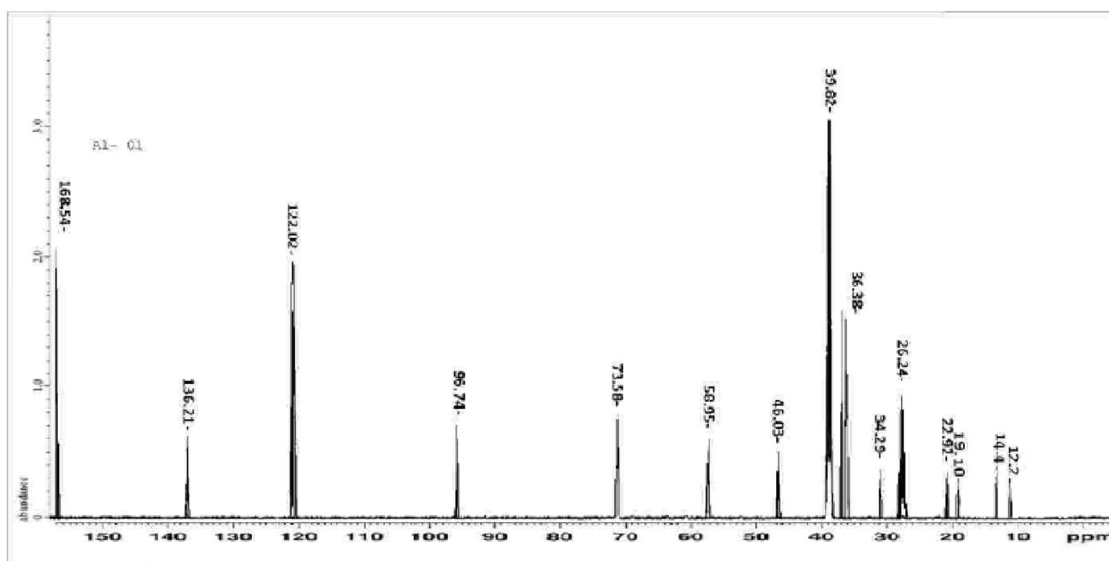
9.2.25: HPTLC of isolated Compound ALL/C1/F4-5/C2/F1-2/comp-01



9.2.26: HPLC of isolated Compound ALL/C1/F4-5/C2/F1-2/comp-01



9.2.29: ^{13}C NMR of isolated Compound ALL/C1/F4-5/C2/F1-2/comp-01



9.3: ANNEXURE III: RESEARCH PUBLICATIONS

1. Prashanthi P, Rajamma AJ, Sateesha SB, Ghosh SK. Pharmacognostical and larvicidal evaluation of *Artocarpus lakoocha* Roxb. from Western Ghats. Indian Journal of Natural Products and Resources. 7(2): 2016; 141-9.
2. Prashanthi P, Rajamma AJ, Sateesha SB, Ghosh SK. Standardization and larvicidal activity of *Cleome viscosa* Linn. Journal of Pharmacognosy and Phytochemistry. 8(1): 2019; 2347-52

9.4. ANNEXURE IV: PRESENTATIONS

9.4.1: ORAL PRESENTATIONS

Sl. no.	Title of Paper	Seminar/ Conference	Year	Remarks
1	Effects of <i>Artocarpus lakoocha</i> Roxb., on growth and development of <i>Anopheles stephensi</i> , <i>Aedes aegypti</i> and <i>Culex quinquefasciatus</i>	International Conference on Medicinal Plants and Drug Discovery ICMPPD, Bengaluru,	July 18-20 th 2018	Best Oral Presentation

2	Herbal biocontrol of mosquito vectors using isolated compounds of <i>Cleome viscosa</i> - A wasteland weed	National seminar on ethnobotany and herbal technology, opportunities and challenges, Karwar.	Mar 18-19 th , 2016	Participation
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9.4.2: POSTER PRESENTATIONS

Sl. no.	Title of Paper	Seminar/ Conference	Year	Remarks
1	Isolation, Characterization, and Formulation of eco-friendly pesticide from <i>Cleome viscosa</i> Linn.	XI ISMODC conference, Bengaluru,	June 11-13 th 2016	Young Scientist Award
2	Isolation and characterization of mosquito larvicidal principles from chloroform extract of <i>Cleome viscosa</i> Linn.	Emerging trends in Biotechnology, Bengaluru.	Feb 20 th 2016	Best poster award
3	Preliminary evaluation of Mosquito Larvicidal Activity of <i>Artocarpus lakoocha</i> Roxb., against three different mosquito species	NIMHANS IPA Convention 2014, Bengaluru.	Aug 8 th 2014	Best poster award

9.4.3: CONFERENCES ATTENDED

Sl no.	Theme of Conference/ Training	Year
1.	Translating Pharmacognosy knowledge into Healthcare Research on Quality and Standards, Bengaluru.	Sept 15 - 16 th 2015
2.	Research Methodology: Importance of biostatistics and technical writing for better understanding of life sciences, Bengaluru.	Sept 14 th , 2015