LARVICIDAL ACTIVITY OF EXTRACTS OF *CARICA PAPAYA* LINN AND *DACROYDES EDULIS* (G.DON) H.J LAM ON THREE MOSQUITO SPECIES

BY

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NIGERIA

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY IN PARTIAL FUFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY OF SCIENCE (PH.D.) IN BIOCHEMISTRY

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JULY, 2017

DECLARATION

I declare that the work in this dissertation entitled "Larvicidal activity of extracts of *Carica papaya* Linn and *Dacroydes edulis* (G.Don) H.J Lam on three mosquito species" has been performed by me in the Department of Biochemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

__________________________	_____________	__________
Halima Temi Akande-Grillo	Signature	Date

CERTIFICATION

This dissertation entitled **LARVICIDAL ACTIVITY OF EXTRACTS OF *CARICA PAPAYA* LINN AND *DACROYDES EDULIS* (G.DON) H.J LAM ON THREE MOSQUITO SPECIES** by **HALIMA TEMI AKANDE-GRILLO** meets the regulations governing the award of degree of Doctor of Philosophy of Science in Biochemistry of the Ahmadu Bello University, and is approved for its' contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to my entire family. Your support has been boundless. Thank you.

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ABSTRACT

Mosquitoes are the number one vector involved in the transmission of human diseases responsible for human debility and mortality. Crude extracts of *Carica papaya* (CP) and *Dacryodes edulis* (DE) have been indicated as having effects on larval mortality. This work was designed to evaluate the larvicidal activities of three solvent extracts and fractions of the seeds, leaves, stems and roots of both plants against the larvae of *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* by standard methods. A standard method was also adopted to determine the growth inhibitory and acetylcholinesterase inhibitory activities. Their effects on a non-target organism were determined and structural analyses of their active principle(s) by FTIR, GCMS and NMR were carried out. Preliminary studies on the crude extracts at 500ppm showed the ethanol and hexane extracts of the seeds and leaves of CP and DE had significant ($p<0.05$) larvicidal activites against the larvae of all three species. Extracts with mortality above 70% against at least two of the three species were assayed for their LC_{50} and LC_{90} values with concentrations of 6.25-400ppm and showed time-dependant increases in activity at 12h, 24h and 48h. The ethanol extract of the seeds of CP gave LC_{50} values of 100.00, 62.24 and 262.14ppm at 48h against the larvae of *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* respectively. The corresponding values for the hexane extract of the seeds of CP were 61.47, 111.76 and 264.78ppm. The ethanol and hexane extracts of the leaves of CP gave LC_{50} values of 93.19 and 48.14ppm against *Aedes vittatus* while against *Anopheles gambiae* the values were 40.80 and 29.32ppm. The LC_{50} values of ethanol and hexane extract of CP against *Culex quinquefasciatus* were 43.96 and 74.83ppm.The ethanol extract of the seeds of DE had the lower LC_{50} value of 150.54, 85.99 and 110.18ppm against the larvae of *Aedes vittatus*,

Anopheles gambiae and *Culex quinquefasciatus* when compared to the hexane extract of the leaves with values of 1177.00, 1762.10 and 508.28ppm against the three species at 48h. The hexane extract of the leaves of CP and the ethanol extract of the seeds of DE were fractionated using column chromatography and the fractions were tested for larvicidal activity against *Aedes vittatus* and *Culex quinquefasciatus. Carica papaya* hexane leaf extract (CPHLE) yielded twelve (12) fractions and fraction 1 had the best larvicidal activity against both species with LC_{50} values of 10.72 and 15.15ppm while *Dacryodes edulis* ethanol seed extract (DEESE) yielded seven (7) fractions and fraction 1 (f1) had the best larvicidal activity against both species with LC_{50} values of 10.48 and 10.16ppm against *Aedes vittatus* and *Culex quinquefasciatus* respectively. Phytochemical analyses of the partially purified CPHLE fraction 1 (CPHLE-f1) indicated the presence of carbohydrates, cardiac glycosides and triterpenes while the partially purified DEESE fraction 1 (DEESE-f1) showed the presence of carbohydrates, cardiac glycosides, steroids, triterpenes, tannins and alkaloids. Growth inhibitory activity of the fractions were evaluated on first, second, early third and late third-early fourth instars of both *Aedes vittatus* and *Culex quinquefasciatus* larvae. CPHLE-f1 gave inhibition values that increased with age of instar. Acetylcholinesterase inhibition was highest with values >30% at a concentration of 100ppm of CPHLE-f1 against both species while DEESE-f1 had the highest inhibition at 6.25ppm with values >10% against *Aedes vittatus* and >20% at 12.5 and 25ppm against *Culex quinquefasciatus*. Effects of the CPHLE-f1 and DEESE-f1 on *Poecelia reticulata* (guppy fish) showed no significant difference between the mortality of tests and controls. Fourier transform infra-red spectroscopy of CPHLE-f1 showed presence of functional groups for alcohol (O-H), alkane/alkyl (C-H), carbonyl (C=O), nitro compound (N-O) and

alkene (C=C) while that of DEESE-f1 showed the presence of alkane/alkyl (C-H), aldehyde (C=O), nitro compound (N-O), acyl/phenyl (C-O), alkoxy (C-O) and alkene (C=C). Gas chromatography mass spectroscopy analysis of CPHLE-f1 showed the presence of eleven (11) compounds while DEESE-f1 showed presence of seven (7) compounds. Column chromatography of CPHLE-f1 yielded five (5) sub-fractions of which sub-fraction 3 provided the highest larvicidal activity. Further purification of the CPHLE-f1 sub-fraction 3 by gel filtration yielded two bands; *fraction* 1 and *fraction* 2 which gave LC_{50} values of 3.9 and 2.25ppm respectively against *Aedes vittatus*. Column chromatography of DEESE-f1 yielded four (4) sub-fractions with larvicidal activity between 31.38 and 94.43ppm. Nuclear magnetic resonance of *fraction* 2 of CPHLE-f1 sub-fraction 3 is suggestive of an eighteen (18) carbon fatty alcohol compound Octa-dec-9-enol. In conclusion, *Carica papaya* and *Dacryodes edulis* contain larvicidally active principles against *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefaciatus* with values that suggest their candidacy for field trials.

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ABBREVIATIONS, GLOSSARIES AND SYMBOLS

ABTS – 2,2□- azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)

ALT – Alanine transaminase

AP- Alkaline phosphatase

AST- Aspartate transaminase

CC = Column Chromatography

CP = *Carica papaya*

CPELE = *Carica papaya* Ethanol Leaf Extract

CPESE = *Carica papaya* Ethanol Seed Extract

CPHLE = *Carica papaya* Hexane Leaf Extract

CPHSE = *Carica papaya* Hexane Seed Extract

^{0}C = Degrees Centigrade

DE = *Dacryodes edulis*

DEESE – *Dacryodes edulis* Ethanol Seed Extract

DEHLE – *Dacryodes edulis* Hexane Leaf Extract

DPPH – 2,2- diphenyl -1- picryhydrazyl

FTIR = Fourier Transform Infra-Red Spectroscopy

GCMS = Gas Chromatography Mass Spectroscopy

Hr = hour

IC_{50} = Concentration at which 50% inhibition is expected

kDa = Kilo Dalton

LC_{50} = Concentration at which 50% mortality is expected

LC_{90} = Concentration at which 90% mortality is expected

µl = Microlire

mg = Milligram

Mg/L – Milligram per litre

NMR = Nuclear Magnetic Resonance

% = Percentage

PPM – Parts per million

SDS – Sodium dodecyl sulphate

SOD – Superoxide dimuase

TLC = Thin layer Chromatography

CHAPTER ONE

1.0 INTRODUCTION

1.1 Overview

Mosquitoes are an ancient group of insects which have persisted for millions of years. Through the process of evolution, nature has superbly perfected them that they may survive under the most adverse and diverse of environmental conditions (Manimegalai and Sukanaya, 2014). Mosquitoes have been a problem for people all over the world as long as humans have existed. They are the most nuisance creatures of nature and the most medically important arthropod vectors of diseases to humans (Beernste *et al*., 2000; Arti *et al.,* 2012). They are responsible for the transmission of dengue, malaria, yellow fever, filariasis (Ali *et al*., 2013), hemorrhagic fever, Japanese encephalitis and other diseases which are today among the greatest health problems in the world. Mosquitoes contribute to social debility and poverty in tropical countries (Rajkumar and Jebanesan, 2005; Alam *et al*., 2011).

Tropical areas are more susceptible to parasitic diseases and the risk of contracting arthropod borne illnesses is increasing due to climate change and rising temperatures worldwide. Trade and unplanned urbanization have also contributed to the impact of disease transmission in recent years. Mosquitoes have been declared public enemy number one by the World Health Organization and they belong to three genera; (anopheles, culex and aedes) responsible for the transmission of diseases to more than 700 million people annually and are responsible for one (1) death in every seventeen (17) people alive (El-Bahnasawy *et al.*, 2013).

Although an ancient and historical disease, malaria persists in many parts of the world today (Hall and Fauci, 2009). Malaria causes human mortality single-handedly killing nearly 3 million people yearly. Malaria fever is one of the deadliest diseases ravaging Africa (Ubulom *et al.,* 2012). It is estimated to have killed between 537,000 and 907,000 in 2010 alone, 86% being children under the age of five with more than 40% of the world's population living in malaria endemic areas (Massebo *et al*., 2009) and an estimated 3.3 billion people in 109 countries are at risk of contacting this serious and often life threatening disease (Hall and Fauci, 2009; Alam *et al*., 2010). There were between one hundred and forty nine million (149,000,000) and two hundred and seventy four million (274,000,000) cases of malaria reported in 2010 with 81% in the African region and there are reportedly between two to three million new cases every year (Kamaraj *et al,* 2011). Malaria kills a child in Africa every 30 seconds and greatly contributes to anemia in children (Nyarko and Cobblah, 2014).

Lymphatic filariasis is transmitted by the bite of infected *Culex quinquefasciatus* mosquito and causes disfigurement and incapacitation. It is estimated that currently there are over 120 million people affected by the disease and 44 million having chronic manifestation (Kamaraj *et al*., 2010). In Nigeria it is believed that two (2) out of every three (3) people are at risk of lymphatic filariasis.

Culex quinquefasciatus is also responsible for the spread of West Nile Virus which has caused repeated epidemics in the continent of North America (Kilpatrick *et al*., 2006). It is considered the most dominant disease in North America.

Dengue and Dengue hemorrhagic fever also called break bone fever is primarily spread by members of the aedes species. There are approximately 50 million dengue infections

annually worldwide with 500,000 individuals being hospitalized with dengue hemorrhagic fever (Guzman *et al.,* 2010). Dengue is endemic in 112 countries (Malavige *et al*., 2004). It is estimated that 2.5 billion people worldwide are at risk of infection (Kean *et al*., 2014) with about 975 million living in South East Asia, the Pacific and the Americas however race appears to affect susceptibility to Dengue (Rogers *et al*., 2006). Fatality with Dengue hemorrhagic fever (DHF) and Dengue shock syndrome (DSS) is as high as 44% (Ukey *et al*., 2010).

The latest addition to mosquito transmitted diseases of medical importance is the Zika virus. This virus is of the family flaviviridae spread by members of the aedes family specifically *Aedes aegypti* and *Aedes albopictus*. It was first discovered in Uganda in 1947 in monkeys and later discovered in humans in 1952 in both Uganda and Tanzania. The first outbreak was reported in 2007 in Micronesia and the latest outbreak in July of 2015 was reported in Brazil with over 1.4millon cases (Jamil *et al*., 2016). The latest outbreak resulted in over four thousand (>4000) children born with fetal brain abnormalities attributed to the Zika virus in Brazil alone (Jamil *et al*., 2016). Due to the latest outbreak Columbia, Dominican Republic, Ecuador, El Salvador and Jamaica asked women to postpone getting pregnant until more is known about the virus.

Yellow fever is a vector borne disease affecting humans and non-human primates in Africa and South America (Garske *et al*., 2014). It is caused by the prototype member of the genus *Flavivirus* (family *Flaviviridae*) (Monath and Vasconcelos, 2015) and is one of the earliest viruses to be identified and linked to human disease (Rogers *et al*., 2006). It is a viral hemorrhagic fever with high mortality that is transmitted by the mosquito. Approximately two hundred thousand (200,000) cases of yellow fever occur annually with 90% of them

occurring in Africa (Rogers *et al*., 2006; Barnett, 2007) and one billion (1,000,000,000) people in 46 countries at risk of contacting it (Barrett, 2016). Though the disease currently occurs only in Africa and South America, the mosquito specie (*Aedes aegypti*) responsible for its transmission occurs in other regions such as Asia.

One of the ways to control mosquito transmitted diseases involves the control of the vectors themselves to interrupt disease transmission (Singh *et al*., 2006). Control of mosquitoes has been achieved mainly by the use of organophosphorus pesticides (OP) (chlorpyrifos, temephos and fenthion), insect growth regulators (diflubenzuron and methoprene, bacterial larvicides and pyrethroids (Govidarajan and Glorintha, 2010; Dong and Young, 2013). Synthetic organic chemical insecticides based intervention for the control of insect pests and insect vectors have disrupted natural biological control systems and led to outbreaks of insect species showing pesticide resistance such as those of malaria (Singh *et al*., 2006) and killed non-target organisms and caused harmful effects on humans (Al-Shaibani *et al.,* 2008) due to their broad spectrum of activity. Mosquito resistance to pyrethroids and DDT has emerged and the potential for carbamate resistance has been detected in *Anopheles arabiensis* in northern Kwazulu-Natal, South Africa (Nardini *et al*., 2013). Synthetic insecticides and their non-biodegradable nature as well as their higher rate of biological magnification through the ecosystem are largely responsible for the increasing insecticide resistance on a global scale (Ghosh *et al*., 2012). It has been observed that acute insecticide poisoning results in double vision, involuntary contraction of muscles and increased lacrimation, dermatitis, liver and kidney disorders and reproductive problems in humans. Carbon compounds from insecticides contribute to global warming and can accumulate in the surface soil layer making farmland infertile. Alternative approaches are

necessary for the development of environmentally safe, biodegradable, cost effective indigenous methods consisting of natural products against vectors. Natural products are preferred because of their less harmful nature to non target organisms and due to their innate biodegradability. Plant products have been used traditionally by human communities against disease vectors and species of insects (Ali *et al*., 2013). The phytochemicals derived from plants can be used as larvicides and insect growth regulators.

Carica papaya belongs to the family Caricaceae. It is called "Ibepe" in Yoruba, "Gwanda" in Hausa and "Okwuru oro" in Igbo. It is native to tropical America. It is a perennial plant and is distributed over the whole tropical area. *C. papaya* fruit is well circulated and is accepted as a food and quasi drug (Kalimuthu *et al*., 2012). Many scientific investigations have been carried out to evaluate the biological activities of various parts of *Carica papaya* including fruits, shoots, leaves, rinds, roots and latex.

Dacryodes edulis is a dioecious shade loving tree belonging to the family Burseraceae. Its common names include African pear, African pear tree, Bush butter, Bush butter tree, Bush fruit tree, Eben tree and Native pear. It is known as safou in French, Ube in Ibo, Elemi in Yoruba, Eben in Efik and Orumu in Benin . It is native to the Gulf of Guinea and Central African countries but it is widely cultivated covering countries like Nigeria, Sierra Leone, Zimbabwe and Angola (Ajibesin, 2011). The tree has a relatively short trunk and a deep dense crown. The bark is pale grey and rough with droplets of resin. The preferred habitat is a shady, humid tropical forest though it is highly adaptive to different soil types, humidity and temperature (Omogbai and Ojeaburu, 2010).

1.2 Statement of Research Problem

Mosquitoes continue to be a problem for human survival and re-emergence of zika virus has reawakened the risk of mosquitoes to the next generation as serious congenital defects in children have been attributed to the virus. Mosquito management programs have not succeeded in controlling the vector without posing hazards to the environment and non target species. Today dissemination of insecticide resistance throughout entire populations is much faster than the rate of development of new insecticides (Rodriquez-Roche and Gould, 2013) and the high cost of these insecticides makes them unrealistic for use in Third World Nations.

1.3 Justification

Larvae are more susceptible to mosquito control efforts because they are concentrated within defined boundaries, are unable to disperse and access to them is relatively easy.

Crude extracts of *Carica papaya* (CP) and *Dacroydes edulis* (DE) have been reported to have mortality effects on larvae however the active principles responsible for larvicidal activity and mode of action of CP are inconclusive and that of DE has not been previously assayed.

This work aims to identify the most effective solvent for the extraction of the active principle of the plants responsible for larvicidal activity; the minimum dose at which activity is present and characterization of the active principles.

1.4 Aim

The aim of this work was to investigate the principles within larvicidally active extracts of *Carica papaya* and *Dacroydes edulis* tested on *Anopheles gambiae*, *Aedes vittatus* and *Culex quinquefasciatus* and to characterize them.

1.5 Specific Objectives

1. To evaluate the aqueous, ethanol and n-hexane extracts of the seed, leaf, stem and root of *Carica papaya* and *Dacroydes edulis* for larvicidal activity.
2. To determine lethal concentrations of the extracts with larvicidal activity.
3. To isolate the effective larvicidal fraction(s) using column chromatography
4. To phytochemically analyze the potent fraction(s) by standard methods
5. To propose a possible mode of action.
6. To determine the effect of active principle on a non target organism of same natural habitat.
7. To structurally evaluate the active principle(s) by IR, GMCS and NMR.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Human Existence and Pesticides

Humans have managed to colonize every corner of the earth and adapted to every habitat. Pests have proven to be dangerous to the very existence of man because they compete with man for food and cause diseases that may shorten lives. Pests destroy crop yield on the farms and reduce harvest both on the farms and in storage (De Lima, 1987) . Due to increases in global population of humans, there is an increasing demand for food production. As such farmers tend to rely on extensive use of pesticides to increase yield (Lu, 2010). In the process of their own survival, pests transmit diseases that have the ability to decimate human populations (Riviero *et al*., 2010). These have resulted in the need for man to devise ways of controlling pests.

Pesticides are substances or mixtures of substances meant for attracting, seducing, destroying or mitigating any pest (Garcia *et al.,* 2012). Though pesticides are more commonly used in terms of plant protection from damaging influences. It has a much broader meaning as they are also used for non agricultural purposes. Pesticides are of vital importance in the fight against diseases, for the production and storage of food being widely used for pest control in agriculture, gardening, homes and soil treatment (Morais *et al*., 2011). They are also useful in that they improve the nutritional value of foods as well as cosmetic appeal which is very important to consumers (Damalas and Eleftherohorinos, 2011). Pesticides can be used to refer to herbicides (used for plant protection), insecticides (which may apply to plant and animal/human protection), bactericide, fungicide as well as disinfectants (Aktar *et al*., 2009). Pesticides can therefore be defined as a chemical or

biological agent such as a bacterium or disinfectant which deters, incapacitates, kills or otherwise discourages pests. Pesticides are classified by target organism, chemical structure and physical state (Garcia *et al.,* 2012). They can also be classified based on their biological mechanism function or application method. It has been suggested that they can be classified from the point of view of acute toxicity, skin irritation, skin toxicity, cummulation coefficient, chronic effect and anticipated chronic risk (Lisaka and Kolesar, 1982). Pesticides can prevent sickness in humans that could be caused by moldy food or diseased produce. Uncontrolled pests such as termites and mould can damage structures such as houses. Without pesticides food prices would soar due to reduced food product (Pimentel *et al*., 1978; Popp *et al.,* 2013). However the contamination of the environment by chemicals is a serious and insidious side effect of human population explosion and technological advancement (Thatheyus and Gnana, 2013).

2.2 History of Pesticides

Pesticide use dates back as far as 2500 BC when sulphur was rubbed on the skin by Sumerians because it was believed that it would help in repelling mites and pests (Taylor *et al*., 2007). Ebers' papyrus of 1550BC recorded the use of over 800 recipes of known substances used as poisons and pesticides. The history of pesticides can be divided into three phases; the first phase being the period before 1870, the second phase being between 1870 and 1945 and the third phase being since 1945 (Zhang *et al*., 2011). The first phase involved the use of natural pesticides, one of the first pesticides ever used being sulfur which was used by the Chinese in around 1000BC to control bacteria and mold. Ancient Romans also burned sulfur as an insecticide and controlled weeds with salt. The Chinese

also pioneered the use of arsenic containing compounds to control insect pests. In the 1600's arsenic and honey were mixed together and used to control ants. Arsenic trioxide was used in the 1800's as a herbicide. By the late 19th century, American farmers were using Paris green (acetoarsenite) to control insect pests in field crops (Taylor *et al.,* 2007). World War II was a turning point in pesticide development as numbers of pesticides such as parathion still in use today were developed as biological warfare during the war (Taylor *et al*., 2007). After World War II, there was an emergence of new pesticides such as DDT, BHC, aldrin and dieldrin. These new chemicals were inexpensive and effective. DDT was enormously popular because it had a wide spectrum of activity against both plant and human pests. However the broad spectrums of activity of these new fangled pesticides are also their undoing because they affected not just the target pests but also non target organisms. This toxicity of pesticides can be direct and indirect (Gibbons *et al*., 2015). Direct toxicity refers to their effects on fish and other crustaceans by killing them outright or exert sub-lethal effects while indirect toxicity refers to the effects of these pesticides on organisms because of their persistence. The persistence of these pesticides are due to their insolubility and the fact that microrganisms have no enzymes capable of degrading them (Pal *et al*., 2006). The effects of indirect toxicity manifests due to bioconcentration and biomagnification. Bioconcentration is the ability for a compound to accumulate in organism tissues while biomagnification is an increase in the concentration as you go up the food chain (Muir, 2012). Due to the emergence of the hazards of indiscriminate use of pesticides, research has been redirected towards more pest specific pesticides and cropping methods that reduce reliance on pesticides. In the 1960's, researchers began developing a different approach to pest control called integrated pest management (IPM) which involves

the reduction but not necessarily the elimination of pests (Alam *et al*., 2016). However, only pesticides can deal with outbreaks also in certain situations any level of pest is considered intolerable especially when the pests can cause physical harm to human and human health. In 2001, over 100 nations signed an international treaty to phase out completely Persistent Organic Pollutants (POP's). Twenty-five (25) developing nations were exempted with their reasons being that the damaging and dangerous effects of presence of pests outweighed the consequences of their usage (Muir, 2012).

2.3 Types of Pesticides

Chronic effects of pesticide exposure include cancer in adults and children and those directly exposed to pesticides are the handlers who mix, load and apply pesticides, and workers cultivating and harvesting crops (Lu, 2010). Mode of action of insecticides are important to enable a better understanding of the health hazards to man and non target organisms, enables the development of other chemicals with similar mode of action and also to have a detailed understanding of the causes of resistance. Most of the insecticides in use are based on the interference of signal transmission with four (4) main targets; acetylcholinesterase, the voltage gated chloride channel, the acetylcholine receptor and the gamma amino-butyric acid receptor (Casida, 2009). Insects depend on the integrated nervous system which enables external stimuli to be translated into effective action (Balkenius and Balkenius, 2016).

2.3.1 Organochlorine insecticides

This was commonly used in the past. Due to their chemical nature and origins, they are classified into four groups:

I. DDT and DDT analogues

II. Lindane and related chlorocyclohexane

III. Cyclodienes

IV. Toxaphene and related compounds

2.3.1.1 DDT and DDT analogues

DDT (Dichlorodiphenyltrichloroethane) is the most popular example of organochlorine insecticides. It was first synthesized in 1874 and at that time it was used for the control of malaria, typhus, body lice and bubonic plague (Garcia *et al*., 2012). The use of DDT was responsible for the drop in the cases of malaria from 400,000 in 1946 to almost none in 1950 in the United States. It is usually sprayed indoors and is considered a contact pesticide. DDT has its effects at a neuromuscular junction brought about by depolarization due to an increased frequency of miniature post-synaptic potentials due to an accelerated spontaneous release of neurotransmitter (Davies *et al*., 2007). If depolarization continues, the neuromuscular junction becomes blocked due to accumulation of neurotransmitter. It prolongs the inward sodium current and also suppresses the increase of the permeability of potassium. A combination of these effects leads to the prolonged falling phase, increases the negative after potential and leads to repetitive activity. The treated insects rapidly become hypersensitive to external stimuli and develop tremors of the body and appendages. After a period of violent motion, they become spasmodic and finally fall into

paralysis (Davies *et al*., 2007). DDT follows the same mechanisms as pyrethroids but the kinetics of the closing of insecticide modified channels is different. It is undesirable as a larvicide because it accumulates in the fatty tissue of humans, fish and other non target organisms. Its use was cancelled because it persists in the environment, accumulates in fatty tissue and causes adverse health effects on wildlife. It is categorized a B2 carcinogen because mammals exposed to it develop liver tumors. Symptoms include CNS excitation and depression, typically abrupt in onset. Initial euphoria with auditory or visual hallucinations and perceptual disturbances with seizures, agitation, lethargy, unconsciousness, cough, shortness of breath, skin rash, nausea, vomiting and diarrhea have also been observed. Chronic exposure is associated with the development of blood dycrasiais, including aplastic anaemia and leukemia (Wong *et al*., 2013).

2.3.1.2 Lindane, Hexachlorohexane (HCH)

It was first synthesized by Michael Faraday in 1825. The insecticide properties were discovered in 1942. It has a strong vaporous action but has a short residual persistence. It is directly absorbed into insects and their eggs. It acts as a stomach poison when it is ingested. In experimental studies, mice fed with lindane had a higher incidence of liver cancer than those untreated (Pavlikova *et al*., 2012). Symptoms include hypersensitivity to stimulation, sensation of tingling, creeping and tingling on the skin also headache, dizziness, nausea, vomiting, lack of coordination, tremor, mental depression, seizures, coma and respiratory depression (Bhat and Ommen, 2013; Ramabnalta *et al*., 2014).

2.3.1.3 Cyclodiene compounds

These were introduced in 1945. Dieldrin is the most popular of this class and is more toxic than DDT and HCH. It is a persistent organic pollutant as it does not break down easily (Cameron and Foster, 2009). It is also biomagnified as it travels through the food chain. It has been strongly linked to the development of Pakinsons disease and breast cancer (GadElhak *et al*., 2010). Symptoms include hypersensitivity to stimulation, sensation of tingling, creeping and tingling on the skin. Other symptoms are headache, dizziness, nausea, vomiting, a lack of coordination, tremor, mental depression, seizures, coma and respiratory depression (Carvalho *et al*., 1991).

2.3.1.4 Toxaphene and related compounds

It is a synthetic organic compound made up of over 670 chemicals it remains in soil and lake sediments for many years (Saleh, 1991; Bonefeld *et al*., 1997). It persists in the food chain and is very toxic. It is harmful to humans and other non target organisms. Toxaphene has a half life of 0.8 year to 14 years in soil (Li and Bidleman, 2001). Since it is easily vapourized, it is even found in regions where it was not used for insect control. Symptoms include seizures, kidney and liver degeneration which affect the nervous system and causes depression of the immune system.

2.3.2 Organophosphate pesticides

Organophosphates affect the nervous system by disrupting acetylcholinesterase, the enzyme that regulates acetylcholine, a neurotransmitter. Most organophosphates are insecticides. The health effects include acting as a nerve agent and causing

organophosphate poisoning in humans resulting in a wide range of exposure because it affects a large number of organs and physical processes (Bajgar, 2004; Bajgar, 2005). Symptoms include miosis (unilateral or bilateral), headache, restlessness, convulsions, loss of consciousness, coma, rhinorrhea (perfuse watery runny nose), bonchorrhea (excessive bronchial secretions), dyspnea (shortness of breath), chest tightness, hyperpnea (increased respiratory rate/depth), bradypnea (decreased respiratory rate), tachycardia (increased heart rate), hypertension (high blood pressure), bradycardia (decreased heart rate), hypotension (low blood pressure), arrhythmias, dysrhythmias (prolonged QT on EKG, ventricular tachycardia, abdominal pain, nausea and vomiting, diarrhea, wheezing, urinary incontinence, frequency, profuse sweating (local or generalized), lacrimation (tear formation), conjunctival injection, weakness (may progress to paralysis), fasciculations (local or generalized) (Singh and Sharma, 2000; Peter *et al*., 2014).

A number of different chemicals have been implicated in transmission at various insect synapses including acetylcholine. Acetylcholine is the transmitter at the central nervous system synapse of insects. In order for the nervous system to operate properly, it is necessary that once the appropriate message has been passed, excess acetylcholine should be removed from the synapse, to prevent repetitive firing and to allow a subsequent message to be transmitted (Callister amd Sah, 1997). This removal is facilitated by the enzyme acetylcholinesterase which calalyses hydrolyses of the ester bond. Organophosphorus and carbamate esters are toxic due to their ability to inhibit acetylcholinesterase (Fukuto, 1990). The enzyme is phosphorylated or carbamylated and the enzyme is no longer capable of effecting hydrolyses of acetylcholine. This results in the

build-up of neurotransmitter at the nerve synapse or neuromuscular junction (Callister and Sah, 1997).

The inhibition of acetylcholinesterase by an organophosphorus ester takes place via a chemical reaction in which the serine hydroxyl moiety in the enzyme active site is phosphorylated in a manner analogous to the acetylation of acetylcholinesterase. The phosphorylated enzyme is highly stable. The serine hydroxylgroup, blocked by a phosphoryl moiety, is no longer able to participate in the hydrolysis of acetylcholine (Chambers and Oppenheimer, 2004; Colovic *et al*., 2013). The ability to interfere with normal nerve impulse transmission makes them toxic to humans and animals.

2.3.3 Carbamate pesticides

They also disrupt acetylcholine; however the effects of carbamate pesticides are usually reversible. Carbamates have a broad spectrum of activity (Mitra *et al*., 2011) thus they can be used as insecticides, fungicides, nematocides, acaracides, molluscicides, sprout inhibitors and herbicides. The mechanism of carbamates except herbicide carbamates involve the carbamylating of the active site of acetylcholinesterase leading to the inactivation of the essential enzyme which has an important role in the nervous system of humans and other animal species (Fukuto, 1990). Acetyl choline accumulates at the cholinergic receptor sites in results in effects equivalent to over stimulation. Herbicide carbamates such as carbetamide work by inhibiting cell division and microtubule organization and polymerization (Vencill *et al*., 2012).

Carbamates are acutely toxic to humans and animals though are not known to be carcinogenic, tetragenic or mutagenic (Risher *et al*., 1987). They present low

bioaccumulation and short term toxicity (relatively short biological half lives) and are fairly rapidly metabolized and excreted. However they are still considered hazardous to the environment and to humans (Morais *et al.,* 2011). Carbamates have been explored as anticancer agent due to its ability to inhibit cellular metabolism including energy, protein and nucleic acid metabolism which results in cellular regression and death (Morais *et al*., 2011).

Clinical symptoms of carbamates are mainly muscarinic symptoms such as miosis, salivation, sweating, lacrimation, rhinnorhea, abdominal cramping, diarrhea, urinary incontinence, bronchospasm, and tachycardia, muscle weakness including respiratory muscles, paralysis, and hypertension (Lifshitz *et al*., 1997).

2.3.4 Pyrethroid pesticides

Pyrethroids are synthetic insecticide derived structurally from the natural pyrethroids found in *Chrysanthemum cinerariaefolium* and *Chrysanthemum cineum.* The six insecticide compounds of pyrethrum are called pyrethrins. Some pyrethroids are toxic to the nervous system. They modify the gating characteristics of neural voltage sensitive sodium channels to delay their closure thereby prolonging neural excitation (Bradberry *et al*., 2005). Many pyrethroids have been linked to distruption of the endocrine system, reproduction and sexual development (Thatheyus and Gnana, 2013). Within the insect they alter the nerves by modifying the kinetics of voltage-sensitive sodium channels, which mediate the transient increase in the sodium permeability of the nerve membrane that underlies the nerve action potential (Soderlund *et al*., 2002).

Since nerve excitation occurs as a result of changes in nerve membrane permeabilities to potassium and sodium ions, the effects of pyrethroids can be seen as a function of those permeabilities. They delay the closing of sodium channels; at the end of an action potential, sodium ions move into the axon and the membrane potential difference becomes more positive, so the negative after-potential is increased. The after potential exceeds the membrane threshold results in another action potential being generated and this continues leading to repetitive activity. This modification results in a disruption of nervous transmission. Pyrethroids also affect other membrane components like the potassium channel (Sonderlund, 2012).

Pyrethroids inhibit calcium, Magnesium ATpase which is believed to be involved in the sequestering of calcium. The inhibition results in an increase in intracellular calcium (Clark and Matsumura, 1986). Symptoms of pyrethroids include sore throat, nausea, vomiting and abdominal pain. Systemic effects are observed between 4-48 hours and are seen as dizziness, headache, fatigue, palpitations followed by coma and possibly death (Bradberry *et al*., 2005).

2.3.5 Suphonylurea herbicides

These are herbicides that act by inhibiting the enzyme acetolactate synthase. Examples include nicosulfuron, triflusulfuron and chlorsulfuron. The enzyme acetolactate synthase is involved in the synthesis of the amino acid valine, leucine and isoleucine (Boldt and Jacobsen, 1998).

2.3.6 Biopesticides

Biopesticides are pesticides derived from natural materials such as animals, plants bacteria and certain minerals. Worldwide there are about 1400 biopesticide products being sold (Chandler *et al*., 2011). Biopesticides are classified into three categories:

I. Microbial pesticides

II. Plant incorporated protectants

III. Biochemical pesticides

2.3.6.1 Microbial pesticides

These consist of micro-organisms for example bacterium, fungus, virus or protozoan as the active ingredient. Microbial pesticides are very specific for its target pest. The most widely used microbial pesticide is *Bacillus thuringiensis* which produces proteins that specifically kill insect larvae such as flies and mosquitoes. The proteins act by binding to the larval gut receptor, thereby causing the insect larvae to starve (Houda *et al*., 2010).

Bacillus thuringiensis israelensis (Bti) is a naturally occurring soil bacterium registered for control of mosquito larvae. Bti was first registered by EPA as an insecticide in 1983. Mosquito larvae eat the Bti product which is made up of the dormant spore form of the bacterium and an associated pure toxin. The toxin disrupts the gut in the mosquito by binding to midgut receptor cells present in insects, but not in mammals causing distruption and insect death (Palma *et al*., 2014). There are 25 Bti products registered for use in the United States. Aquabac, Teknar, Vectobac, and LarvX are examples of common trade names for the mosquito control products. *Bacillus sphaericus* is a naturally occurring bacterium that is found throughout the world. *Bacillus sphaericus* was initially registered

by EPA in 1991 for use against various kinds of mosquito larvae. Mosquito larvae ingest the bacteria, and as with Bti, the toxin disrupts the gut in the mosquito by binding to receptor cells in the larval midgut present in insects (Poopathi and Abidha, 2010). VectoLexCG and WDG are registered *B. sphaericus* products, and are effective for approximately one to four weeks after application (Chakoosari, 2013). The major disadvantages of microbial insecticides are that they are susceptible to degradation by sunlight, they are very specific, have a short shelf life and they kill quite slowly (Opender, 2011; Bonaterra *et al.*, 2012)

2.3.6.2 Plant incorporated protectants (PIP)

These are pesticides products produced by plants with genetic material added to the plant. The gene of a toxic protein can be incorporated into the plant to be protected so it indivertibly kills the pest of that plant (Duan *et al*., 2007).

2.3.6.3 Biochemical pesticides

These are naturally occurring substances that control pests by non-toxic mechanisms. These differ from conventional pesticides which are mostly synthetic materials that directly kill or inactivate the pest. Biochemical pesticides include insect sex pheromones which interfere with mating or insect growth regulators, naturally occurring substances such as plant extracts and fatty acids (Sarwar, 2015).

2.3.7 Botanical pesticides

Botanical pesticides are plants which have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions (Ayvaz *et al*., 2010). Plants have evolved over the last 400 million years to defend themselves from insect attack. They have evolved protection mechanisms such as repellants and even insecticidal effects. After the 2nd World War, all the plants that showed insecticide promise were replaced with synthetic pesticides. However with the advent of environmental contamination and other hazards, interest in botanical pesticides was renewed (Sawmya *et al*., 2012).

The use of plant extracts as insecticides date back as far as the Roman Empire. The first botanical insecticide was nicotine, obtained from tobacco leaves which killed plum beetles. In 1850, a new plant insecticide known as rotenone gotten from the roots of the plant timbo was introduced (El Wakeil, 2013). The use of incense and sabadilla was used to drive insects away though they did not kill them. Pyrethrum is the most widely used commercial biopesticide. It is known to affect insect behavior by causing excite repellency recions (Mann and Kaufman, 2012). It has been used since the 1800s to control body lice in Persia and later to control mosquitoes. Chemically pyrethrum contains six closely related insecticide esters referred together as pyrethrins (Casida *et al*., 1980).

In modern times, there are insecticides being marketed which are extracted from neem, grapefruit seeds and garlic. Primary plant metabolism synthesizes essential plant botanicals that are common to all plants. The end products of secondary metabolism are not universally present in all plants (Grdisa and Grsic, 2013).

Common amongst these metabolites are compounds with protective action against insects such as alkaloids, steroids, phenols, flavonoids, glycosides, glucosinolates, quinines, tannins and terpenoids. These metabolites act as insecticides via different mechanisms such as acting as insect growth regulators, feeding deterrents, repellents and confusants (Rajasheka *et al*., 2012).

2.4 Metabolites with Insecticide Activity

2.4.1 Alkaloids

These are nitrogenous compounds that have insecticidal properties. They are also toxic to vertebrates, example is nicotine. The mode of action of alkaloids is reported to affect acetylcholinesterase and sodium channels (Mann and Kaufman, 2012).

2.4.2 Phenols

Phenols are a class of compounds that contain a hydroxyl group attached to an aromatic hydrogen group (Kabera *et al*., 2014). Within the phenolics, flavonoids and tannins are known to possess insecticidal properties.

2.4.3 Terpenoids

Terpenoids are the most diverse plant pesticide. The triterpinoid from the neem tree such as citronella and pinene are common terpenoids known to possess insecticide properties (Singh and Sharma, 2015).

2.4.4 Essential oils and their constituents

Essential oils are volatile, natural, complex compounds characterized by strong odour and formed by aromatic plants as secondary metabolites (Tripathi *et al*., 2009). They are lipophilic in nature and interfere with basic metabolic, biochemical and physiological and behavioral functions of insects. The majority of essential oils originate in the plant families Myrtaceae, Lauraceae, Rutaceae, Lamiaceae, Asteraceae, Apiaceae, Cupressaceae, Poaceae, Zingiberaceae and Piperaceae. They are classified into four major categories based on their structures (Abdel-Tawab, 2016).

I. Terpenes
II. Benzene derivatives
III. Hydrocarbons
IV. Other miscellaneous compounds

2.5 Larvicides and Larviciding

All larvicides are insecticides but not all insecticides are larvicides. Larvicides are insecticides specifically targeting the larva stage of the insect (Mboera *et al*., 2014). Larviciding is a general term used for killing immature mosquitoes by applying agents generally called larvicides. There are different types of larvicides and they fall under the following general classifications:

2.5.1 Biological agents

Biological control involves the introduction or manipulation of organisms to suppress vector population (Chandra *et al*., 2008). A wide range of organisms help in the

suppression of vector populations naturally through predation, parasitism and competition. *Bacillus thuringiensis* (Bt) falls under the category of biological agent. It is a soil bacterium that forms spores during the stationary phase of the growth cycle. The spores contain crystals comprising of δ-endotoxins that have potent and specific insecticidal activity (Sanahuja *et al*., 2011). Different strains of Bt produce different types of toxin, each which affects a specific taxonomic group of insects. It acts when the spores upon ingestion dissolve in the larval midgut. It is proteolytically converted into a toxic core fragment of 60-70 kDa. The activated toxin interacts with the midgut epithelium cells and feeding stops. The gut wall breaks down and death follows (Deist *et al*., 2014).

Larvivorous fish are another type of biological control of larvae. Since 1937, fish have been used for larvicidal control. Fish of indigenous origin have been found to be more appropriate in larval control. Examples of larvicidous fish include *Poecilia reticulata* and *Gambiesia affinis* (Chandra *et al*., 2008). However larvivorous fish can only be used under certain conditions conducive to their own survival (Howard *et al*., 2007). Other disadvantages of larvivorous fish include that they are less effective in waters with much vegetation or floating garbage and they are only effective in large numbers and have been found to be most effective when combined with other control methods. They will only thrive and reproduce under certain conditions specific for each specie, not all containers that allow for mosquito breeding can accommodate fish; they also cannot be used in habitats that are prone to drying out (Rodriguez-Perez *et al*., 2012).

2.5.2 Contact pesticides

These are compounds effective against larvae and pupae upon contact with them. The chemicals are absorbed through the skin or cuticle and may be incidentally ingested. Contact agents are subdivided into two groups: Those affecting primarily the insects' nervous system and those primarily affecting the insects' endocrine system (Salako *et al.,* 2012).

2.5.3 Insect growth regulator

These are larvicides modeled after natures' insect juvenile hormone (Siddal, 1976). They block naturally occurring ecdysone from initiating the process of molting and inducing metamorphosis in the mosquito larvae. Juvenile hormone is found throughout the larval stages of the mosquito but most prevalent during the earlier instars. It steadily declines until just prior to the 4th instar molt when larvae develop into pupae (Tripathi and Tiwari, 2014; Uryu *et al*., 2015). Hydroxyecdysone (20E) regulates gene transcription via the nuclear receptor ECR to promote metamorphosis. Methoprene (Isopropryl-11-methoxy-3,7,11- trimethyl-2,4,- dodecadienoate) is a terpenoid which mimics the juvenile hormone which regulates gene transcription via its intracellular receptor to prevent larval-pupal transition (Wei-Li *et al*., 2014).

2.5.4 Organophosphates

Temephos is the most popular of the neurological contact larvicides. It was registered by the EPA in 1965. It is an organophosphate which acts by inhibiting acetylcholinesterase activity (Marina *et al*., 2014).

2.5.5 Monomolecular films

These are chemicals that spread a thin film on the surface of the water that make it difficult for mosquito larvae and pupae by closing off their respiratory structures (siphon in larvae and trumpet in pupae) leading to suffocation and prevention of emerging adults from attaching to the water's surface causing them to drown. These films can remain active typically 10-14 days on standing water. There is risk of toxicity to fish, other aquatic animals and non target organisms (Bukhari *et al*., 2011).

2.5.6 Oils

These are gotten from petroleum distillate from petroleum. They are applied to the top (surface) of still water to drown larvae pupae and emerging adults. There is risk of toxicity to fish and other aquatic organisms (Sukkanon *et al*., 2016)..

Generally pesticides are toxic to target and non target organisms via different mechanisms. The environment is also not spared due the perpetuation of some of them. Biopesticides have their limitations and some of the metabolites identified as having insecticidal or larvicidal activity may also be toxic to vertebrates. This necessitates the importance of ensuring that organisms of the ecosystem of the larvae are not adversely disturbed by the application of biopesticides or larvicides thus these larvicides must be tested on non- target organism(s) of the same habitat to ascertain their safety.

2.6 The Mosquito

Taxonomy

Kingdom – Animalia

Phylum - Arthropoda

Class - Insecta

Order –Diptera

Family – Culicidae

Genus – Aedes, Anopheles, Culex

Diptera is one of the largest orders of insects consisting of more than 85,000 species. The predominant group amongst these is mosquitoes (Manimegalia and Sukanaya, 2014). There are approximately 3,500 species of mosquitoes grouped into 41 genera (Nassar *et al*., 2016). The mosquito life cycle is one of complete metamorphosis (Gouge *et al*., 2001). The eggs of some mosquitoes float on the water in rafts. Larvae are often called wrigglers having a soft body but a hard head and a siphon (breathing tube) at the tip of the abdomen. The pupae are shaped like a comma, and are commonly called tumblers. The adults have delicate legs a long proboscis and one pair of transparent wings. Female mosquitoes are usually larger than the males of the same species. Females have fine threadlike antenna with few hairs, whereas the males have bushy antenna. The eggs, larvae and pupae of the mosquito are aquatic (Beck-Johnson *et al*., 2013), while the adults are free flying. The larvae of mosquitoes go through four instars before pupating. The males die within seven (7) days while the female may live for several weeks in presence of adequate sugar source. Host recognition between mosquitoes and human is achieved by the mosquito olfactory

system. Antennal sensilla which houses olfactory receptor neurons are responsible for detecting chemical cues from the hosts (Ye *et al*., 2016). Some mosquito species fly considerable distances while others tend to stay close to larval habitats (Gouge *et al*., 2001).

In addition to the well known diseases that mosquitoes vector, new evidences are now being reported of the relationship between mosquitoes and cancer. The special emphasis being on malaria due to its ability of plasmodium to suppress the immune system. It has been hypothesized that Anopheles vectors may transmit obscure viruses linked with cancer development (Benelli *et al*, 2016).

2.6.1 *Aedes vittatus* (Bigot)

Taxonomy

Kingdom - Animalia

Phylum - Arthropoda

Class - Insecta

Order: - Diptera

Family - Culcidae

Genus - Aedes

Species - *A. vittatus*

The Aedes mosquito originating from Africa is one of the most dangerous type of mosquitoes. There are about 700 species in this genus. Most species can be found mostly in tropical climates but have recently been found in some temperate areas of the world.

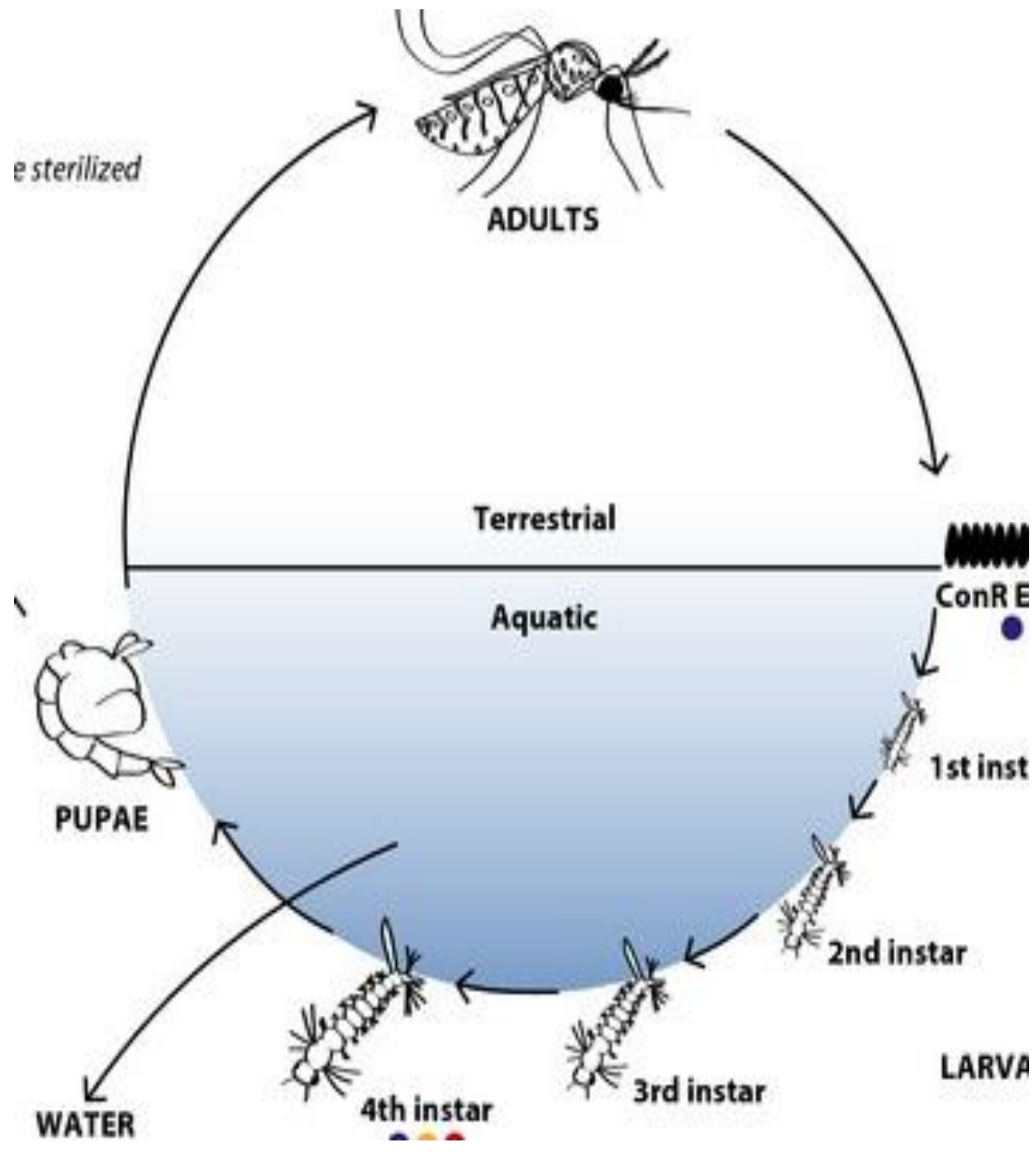

Figure: 2.1 Generalised life cycle of mosquito

Source: Coon *et al*., 2014.

They are responsible for the transmission of dengue, yellow fever, west nile fever, chikungunya and lymphatic filariais (Alikhan *et al*., 2014).

Aedes vittatus is also known as rock pool mosquito. They are very distinct from other mosquitoes by the black and white markings on their bodies and legs. Unlike other mosquitoes, they are active and bite only during the daytime commonly during dusk and dawn, indoors in shady areas and are capable of spreading infection throughout the year. They are fresh water breeding mosquitoes thus they are very difficult to control during the rainy season (Hayatie *et al*., 2015). They produce average of 100-200 eggs singly but can produce up to 500 eggs. The amount of eggs produced is dependent on the size of the blood meal. The eggs are white when laid then turn shiny black. *Aedes vittatus* eggs can survive desiccation for up to nine months and hatch after being submerged in water. The life cycle of this species is completed between 1 ½ and 3 weeks. The various diseases transmitted by Aedes species occur when female mosquitoes take a blood meal from an infected person. The viruses spread by this species of mosquitoes are typically accompanied by fever which can lead to death. The viruses pass from the mosquito intestinal tract to the salivary glands after an incubation period of seven (7) to ten (10) days and become infective and mosquito bites after this period results in infection. During the incubation period the virus replicates and reaches the salivary glands of the mosquito then the infections are passed on when the infected mosquito takes another blood meal (Guzman *et al.,* 2010).

Dengue is an arbovirus infection with significant morbidity and mortality. The severity of dengue shock syndrome (DSS) is age dependent with the worst cases observed in children. The symptoms of the disease include fever, headache, muscle and joint pains and a characteristic skin rash similar to measles. Dengue haemorrhagic fever and dengue shock

syndrome are characterized by increased vascular permeability, plasma leakage and hypovolemia. The disease is caused by four (4) closely related dengue viruses (DENV1-4) (Nishiura and Halstead, 2007). The dengue viruses belong to the genus flavivirus within the *Flaviviridae* family. Other members of the family are the yellow fever virus, west nile virus, the chikungunya virus and rift valley virus. All these arbo viruses are primarily transmitted by the aedine species (Kean *et al*., 2014). Dengue infection can be life threatening in patients with asthma, diabetes and other chronic diseases (Pang *et al*., 2017). Zika virus also transmitted by members of the aedes family has symptoms similar to a mild form of dengue with fever, skin rash, conjunctivitis, muscle joint pain, malaise and headache lasting 2-7 days. Apart from the spread by the bite of an infected mosquito, it can also be transmitted by the bites of monkeys and through sexual transmission (Jamil *et al*., 2016). Transmission from infected pregnant mother to child results in birth defects (Driggers *et al.,* 2016) such as microcepnaly and severe brain malformations (Johansson *et al*., 2016).

Currently immunization is the only way of prevention of yellow fever. Transmission in Africa is maintained by a high density of vector mosquito populations that are in close proximity to largely unvaccinated human populations (Barnett, 2007). Though an effective vaccine has been available for over 70 years the disease remains a threat due to the massive unprecedented mobility of people within endemic areas (Monath and Vasconcelos, 2015). In the 1990s despite massive vaccination it was discovered that in rare cases the vaccine can cause a disease similar to parental wild type virus (Monath, 2001). In humans the symptoms vary and depend on severity of infection. Between days three-six after infection influenza like symptoms are observed such as fever, joint pains and headache (Rogers *et*

al., 2006). On the fourth day of infection in a few cases symptoms disappear and the victim recovers but in the majority of cases after remission of 6-12 hours other symptoms appear such as nausea, vomiting, epigastric pain, hepatitis with jaundice, renal failure, hemorrhage, shock in 20-60% of cases observed with death occurring within 10-14 days (Monath, 2001).

2.6.2 *Anopheles gambiae* (Giles)

Taxonomy

Kingdom - Animalia

Phylum -Arthropoda

Class -Insecta

Order - Diptera

Family - Culicidae

Genus -Anopheles

Species - *A.gambiae*

Anophelines are found worldwide except in Antarctica. Human malaria is transmitted primarily by the females of the genus *Anopheles.* About sixty (60) members of the Anopheline species out of about four hundred and sixty (460) are vectors of malaria (Sokhna *et al*., 2013). Anophelines are also responsible for the transmission of filariasis. Malaria is an acute febrile illness mainly transmitted by mosquitoes of the *Anopheles gambiae* complex, *Anopheles funestus* group, *Anopheles nili* complex, and *Anopheles moucheti* complex (Sokhna *et al*., 2013). *Anopheles gambiae* complex includes the two

primary vectors *Anopheles gambiae s.s* and *Anopheles arabiensis* of human malaria in sub-Saharan Africa (Ahmed and Ahmed, 2011). They are responsible for an estimated 240 million cases and 280,000 deaths worldwide with over 80% occurring in Africa. These two species are the most wide-spread of the *Anopheles gambiae* complex and are major vectors of malaria (Nghabi *et al*., 2011). *Anopheles gambiae s.s* is found in more humid climates while *Anopheles arabiensis* has greater tolerance for drier environments (Drake and Beier, 2014)

The first symptoms appear about seven days after the infective mosquito bite but typically 8-25 days though symptoms may occur later in people who have taken anti-malarial medication as prophylaxis. The symptoms can resemble those of the flu, gastroenteritis, septicaemia and viral infections and they include fever, headache, chills, vomiting, arthralgia and jaundice (Bartolini and Zammarchi, 2012). If not treated within 24 hours it can progress to severe illness often leading to death. In children the symptoms may include severe anemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria. In endemic areas, persons may develop partial immunity resulting in asymptomatic infections. Due to the non-specific nature of the disease presentation, diagnosis in non-endemic countries requires a high level of suspicion which might be elicited by any of the following; recent travel, history, splenomegaly (enlarged spleen), fever, thrombocytopenia, and hyperbilirubinemia combined with a peripheral leucocyte count. Malaria is caused by blood parasites (protists) transmitted from person to person through the bites of infected female Anopheles mosquitoes (Bartolini and Zammarchi, 2012). The transmitted parasites responsible for the disease are of four species:

I. *Plasmodium falciparum*

II. *Plasmodium vivax*

III. *Plasmodium malariae*

IV. *Plasmodium ovale*

Plasmodium falciparum and *Plasmodium vivax* are the most common with *Plasmodium falciparum* the most deadly (Clark, *et al.,* 2004). In recent years, some human cases of the infection have occurred with the specie *Plasmodium knowlesi.* The bite of the mosquito introduces the saliva containing the protists into the circulatory system, and ultimately to the liver where they mature and reproduce (Soulard *et al*., 2015).

The female mosquito gets the malaria causing parasite from an infected host when it takes a blood meal for egg development. The parasite passes through several developmental stages within the female mosquito (Cator *et al*., 2014). During the stages of maturation (extrinsic incubation period), the mosquito shows decreased interest in biting and blood feeding, however the intensity of feeding increases upon the maturation of the parasites in the gut of the female mosquito and transference to the salivary glands (Fergusen and Read, 2004). The parasites are released into the blood stream of the new host upon the taking of another blood meal by the mosquito (Lacroix *et al*., 2005). Both male and female mosquitoes feed on nectar and damaged fruits. The female anopheles mosquito lays about 50-200 eggs in batches directly into water. In the tropical climate the eggs hatch within three (3) days. After hatching the eggs become larvae that are equipped to feed. The larvae of the anopheles mosquito do not have a respiratory siphon hence this necessitates their being parallel to the water surface at all times. Larvae develop in four (4) instars and the end of which they metamorphose into pupae during a five minute process. The pupa is

comma shaped and the least active of the anopheles cycle. It requires frequent visits to the water surface at all times to breathe. After 2-4 days the pupa metamorphoses into an adult which is ready to fly within minutes. Mosquitoes mate during flight. The male is attracted to the female by the tone of her wing beat and it has antenna that act as receptors. After mating the female seeks out a host for a blood meal. The duration from the egg to the adult stage in the average mosquito is 10 -14 days. Males of the specie live for about one (1) week and the females live for between one to two (1 – 2) weeks in nature. The female feeds on sugar sources for energy but require a blood meal for egg development (Robinson, 2013). After taking a blood meal the female rests for a few days to allow for digestion and egg development.

2.6.3 *Culex quinquefasciatus* (Say)

Taxonomy

Kingdom - Animalia

Phylum - Arthropoda

Class - Insecta

Order - Diptera

Family - Culicidae

Genus - Culex

Species - *C. quinquefasciatus*

Culex quinquefasciatus is an obligatory ectoparasitic vector (Manimegalai and Sukanaya, 2014). There is evidence of the fact that the mosquito feeds on birds as well as humans

making them excellent vectors of avian pathogens and bridge vectors (Uttah *et al*., 2013) capable of infecting humans and other animals (Wan-Norafikah *et al*., 2013a).They are vectors of filarial worms, protozoan parasites and various arboviruses (Gokhale *et al*., 2013) and as such are responsible for the transmission of West Nile Virus, Filariasis, Japanese Encephalitis, St Louis Encephalitis and Avian Malaria.

This mosquito is commonly known as southern house mosquito. It is found in Africa, North America, South America, New Zealand, Asia and Australia. These mosquitoes proliferate and spread very effectively, this may be due to their short life cycle of between 10-14 days, the onetime insemination and high prodigality of production (Manimegalai and Sukanaya, 2014). They are brown in colour and they lay their eggs on the surface of fresh or stagnant water. They have preference for water that is sheltered from the wind by grass and weeds. The eggs look like soot on the surface of the water. The eggs are laid at night in rafts loosely cemented together. The eggs hatch within 24-36 hours after ovipositioning. The larvae from the hatched eggs feed on biotic materials and complete their development between 5-8 days at 30°C. The larvae of Culex must come to the surface frequently to take oxygen through a breathing tube called a siphon attached to the larval abdomen. They develop through 4 instars shedding their outer skin before molting into pupae. The pupae live between 1 and 4 days depending on temperature before the adults emerge (about 36 hours at 27°C) the pupae take oxygen through two breathing tubes called trumpets. Pupae do not eat. The pupae case splits and the adult emerges to the surface of the water and rests for its body to dry and harden. Both males and females feed on plant nectar. Only female mosquitoes bite because of the need for a blood meal for proper egg development. In humans the preferred biting point is the foot area (Uttah *et al*., 2013). A female can lay up

to five rafts in its lifetime. The exact number of eggs produced in each raft is dependent on climatic conditions. The eggs of *Culex quinquefasciatus* are not dessication resistant. The adults do not usually disperse greater than one kilometer from a hatching point and stay close to breeding habitat and host sources. In urban areas they thrive in wet pit latrine, blocked open drains and polluted puddles (Yadouleton *et al.,* 2015).

Lymphatic filariasis infection involves asymptomatic, acute and chronic conditions. The majority of infections are asymptomatic, showing no external signs of infection but still cause damage to the lymphatic system. Acute episodes of local inflammation involving skin, lymph nodes and lymphatic vessels often accompany the chronic lymphoedema (tissue swelling), elephantiasis (tissue thickening) and hydocele (fluid accumulation) (Nutman, 2013). Some of these symptoms are caused by the body's immune system reacting to the presence of the parasite however some are the result of bacterial skin infection (Addiss and Brady, 2007; Shenoy, 2008) *Culex quinquefasciatus* carries nematodes of the family filariodidea. There are three types of these thread-like filarial worms.

I. *Wuchereria bancroftti* which is responsible for 90% of cases
II. *Brugia malayi*, which causes most of the remainder of the cases
III. *Brugai timori* which also causes the disease

Adult filarial worms lodge in the lymphatic system and disrupt the immune system. They usually live for 6-8 years during their lifetime and produce millions of microfilariae that circulate in the blood. Lymphatic filariasis is common in tropical and subtropical regions with an estimated 120 million people in 81 countries are infected, a third of who have overt clinical disease. About one-third of those infected reside in India, a third in Africa, and the

remainder in the Americas, South-East Asia and the Pacific, including many of Australia's neighbours' and countries with which Australia shares strong migration links (Jeremiah *et al.,* 2011).

Culex quinquefasciatus is also responsible for the transmission of the west nile virus. There are no medicines or vaccines for the disease and most people will have no symptoms; however 20 to 30% people will develop flu like symptoms (Samuel and Diamond, 2006) such as fever, headache, body aches, nausea, vomiting, swollen lymph glands, rash on stomach or back and 1% of those who become symptomatic will develop serious fatal neurologic symptoms such as fever, confusion, convulsions, muscle weakness, vision loss, numbness, paralysis, coma and finally death (Madden, 2003). The virus is transmitted by the mosquitoes which have bitten infected birds (Paz, 2015). It is also sometimes transmitted via blood transfusion and solid organ transplants (Blau *et al*., 2013).

Currently immunization is the only way of prevention of yellow fever. Transmission in Africa is maintained by a high density of vector mosquito populations that are in close proximity to largely unvaccinated human populations (Barnett, 2007). Though an effective vaccine has been available for over 70 years the disease remains a threat due to the massive unprecedented mobility of people within endemic areas (Monath and Vasconcelos, 2015). In the 1990s despite massive vaccination it was discovered that in rare cases the vaccine can cause a disease similar to parental wild type virus (Monath, 2001). In humans the symptoms vary and depend on severity of infection. Between days three-six after infection influenza like symptoms are observed such as fever, joint pains and headache (Rogers *et al*., 2006). On the fourth day of infection in a few cases symptoms disappear and the victim recovers but in the majority of cases after remission of 6-12 hours other symptoms appear

such as nausea, vomiting, epigastric pain, hepatitis with jaundice, renal failure, hemorrhage, shock in 20-60% of cases observed with death occurring within 10-14 days (Monath, 2001).

2.7 *Carica papaya*

Taxonomy

Kingdom - Plantae

Order -Brassicales

Family -Caricaceae

Genus -Carica

Species - *C. papaya*

Carica papaya is a member of the family caricaceae. It is a dicotyledonous, polygamous and diploid species. It has its origins in Sothern Mexico, Central America and the Northern part of South America. It is now cultivated in many tropical countries (Soobitha *et al*., 2013). The annual global production of *Carica papaya* is placed at over 100 million tonnes (Agunbiade and Adewole, 2014). It is a tree reaching 3-10m in height The fleshy stem is marked with scars where leaves have fallen off. This is surmounted by a terminal panache of leaves on long petioles with 5-7 lobes (Krishna *et al*., 2008). It is rich in Vitamin C, Vitamin E and Vitamin A, panthotenic acid, folate, B vitamins, magnesium, sodium, phosphorus, iron, calcium and potassium. Extracts of *Carica papaya* contain terpenoids, alkaloids, flavonoids, carbohydrates, glycosides, saponins and steroids (Aravind *et al*., 2013). *Carica papaya* is a neutroceutical plant having a wide range of pharmacological

activities (Jyotsna *et al*., 2014). It contains many protelolytic enzymes such as papain an enzyme similar to pepsin and chymopapain and is effective in the treatment of slipped disc (Benoist *et al.,* 1982). Chymopapain and papain are widely used for digestive disorders (Aravind *et al*., 2013). The alkaloid, carpaine, slows the heart rate in humans and thus reduces blood pressure which is similar in action to digitalis (Aravind *et al*., 2013). Carpaine is also used to kill worms and amoebas. Externally the papaya latex is an irritant to the skin and internally it causes severe gastritis. The leaves of *Carica papaya* have been shown to contain many active components such as papain, chymopapain, cystalin, tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides and glucosinolates (Seigler *et al*., 2002) that can increase the total antioxidant activity in blood and reduce lipid peroxidation level. Leaves have been used by tropical tribal communities as poultices for nervous pains and are smoked for the control of asthma and the leaf juice extract is used in the Gold Coast of Australia for its anticancer activity (Nguyen *et al*., 2016). Dried and pulverized leaves are also used as a purgative for horses (Ahmad *et al*., 2011). Hypoglycaemic effect of the extract of unripe mature fruits has been reported (Natarajan, 2014). Seeds and fruits are excellent anti-helmithic and anti-amoebic (Gunde and Amnerkar, 2016). The seeds are also regarded to have contraceptive properties (Chinoy *et al*., 1995; Panday *et al*., 2016). Some people are allergic to various parts of the fruit and even the enzyme papain has its negative properties. Most notable is its ability to induce asthma and rhinitis and its sister enzyme carpaine can cause paralysis, numbing of the nerve centers and cardiac depression (Novay *et al*., 1979; Quinones *et al*., 1999; Goeminne *et al*., 2013; Kantham, 2013).

Plate I: Picture of *Carica papaya* (pawpaw)

2.7.1 Research based medicinal uses of *Carica papaya*

2.7.1.1 Anti-bacterial and antifungal activity

Alcohol extracts of *Carica papaya* fruits were found to be effective against wound cultures of *Staphylococcus aureus*, *Enterococcus faecalis*, *Eschericha coli*, *Pseudomonas aeruginosa* and proteus species (Akujobi *et al.*, 2010). Seed extracts inhibited gram positive and gram negative bacterial organisms (Dawkins *et al*., 2003) with the aqueous extract having increase in inhibition against *Staphylococcus aureas, Pseudomonas aeruginosa* and *Eschericha coli* with increase in concentration (Jyotsna *et al*., 2014). The methanol extracts of the leaves showed greater inhibition against *Staphlococcus aureus* than *Eschericha coli* and *Candida albicans* (Gomathinayagm *et al*., 2014). The leaf extracts were effective against gram negative bacteria especially *Pseudomonas aeruginosa* (Anibijuwon and Udeze, 2009). It has also shown activity against *Salmonella typhi* with IC_{50} values of 18.38mg/L (Yismaw *et al*., 2008). Acetone, aqueous, ethanol and methanol leaf extracts of *Carica papaya* were tested against *Bacillus subtilis, Clostridium tetanus, Escherichia coli, Proteus vulgaris, Stapylococcus aureus* and all extracts were shown to have activity against all species tested showing broad spectrum of activity (Vijayakumar *et al*, 2015).The latex has been found to have synergistic activity with fluconazole against *Candida albicans*. A chitinase gene has also been cloned from the fruit of the plant. This gene has been classified as a class IV chitinase based on its amino acid sequence homology with other plant chitinases (Krishna *et al*., 2008). Aqueous seed extract was tested against Rhizopus spp, Aspergillus spp and Mucor spp and was found to have remarkable mycelial inhibition against all three species (Nwinyi and Abikoye, 2010). The extracts of the root, shoot and seeds have also been found to have a positive concentration based inhibition

against *Aspergillus flavus*, *Candida albicans* and *Calatropic procera* (Kumar *et al.*, 2013). When the Maradol leaves and seeds of *Carica papaya* were tested on Fusarum spp and *Collectotrichum gloeosporiodes* the leaf extract was found to have the broadest spectrum of activity (Chavaz-Quintal *et al.,* 2011).

2.7.1.2 Anti-tumor activity

Several parts of *Carica papaya* has been tested for its tumor inhibition activity. Leaf extract of *Carica papaya* is shown to have significant growth inhibitory activity on tumor cell lines (Otsuki *et al*., 2010). It is effective in boosting key signaling molecules called Th1-type cytokines, which help regulate the immune system in cancer cell inhibition (Aravind *et al.,* 2013). Lycopene extracted from the plant was able to inhibit the proliferation of liver cancer cell line HepG2 with IC_{50} value of 22.8g/ml. N-hexane extract of the seed dose dependently inhibited superoxide generated in acute promyelotic leukeamia HL-60 cells with IC_{50} values of 10g/ml (Ganeia-Solis *et al.,* 2009). The aqueous extract of the flesh and the ethanol extract of the pericarp have been shown to significantly ($p<0.05$) inhibit the effects of the proliferation of MCF-7 breast cancer cells (Ganeia-Solis, 2009; Jayakumar and Kanthimathi, 2011). Aqueous extract of the leaves showed a concentration dependent anti cancer effect on pancreatic cancer cell line Capan-1, colon cancer DLD-1 cell line, lymphoma cell line Kapan, ovarian cancer Dov-1 cell line, stomach cancer AGS cell line, breast cancer cell line MCF-7, neuroblastoma T98G cell line and uterine cancer cell line Hela (Yogiraj *et al*., 2014). Another research on the aqueous extract of the leaves resulted in inhibition of proliferation responses of both haemopoetic cell lines and solid tumor cell lines. In peripheral blood mononuclear cells the extract reduced the

production of IL-2 and IL-4 and increased production of Th1 type cytokines such as IL-12p40 (Otsuki *et al*., 2010). Benzyl glucosinolate has been indicated in anti tumour growth and found in higher quantities in the seed than in the leaves upon maturation of the plant and shown to have anti proliferation activity against human lung cancer H69 cell (Li *et al*., 2012) while papain in another study showed an IC_{50} value of 125µg/ml with 80% cytotoxicity at 1000µg/ml against HepG2 liver cell line (Akila *et al.,* 2014).

2.7.1.3 Antimalarial and anti-plasmodial activity

Different parts of the plant have shown antimalarial activity to varying degrees. The petroleum ether extract of the seed rind of *Carica papaya* has shown considerable anti-malarial activity with an IC_{50} in µg/ml (Vincent *et al*., 2008). Using the same solvent to extract the rind and pulp the extracts were able to produce a change in the morphology and number of parasites with IC_{50} values of 15.16 and 18.09µg/ml respectively (Bhat and Surolia, 2001). The assay of the ethanol leaf extract gave IC_{50} values of 40.75, 36.54, 25.30 and 18% in chloroquine sensitive strains and 50.23, 32.50, 21.45 and 23.12% in chloroquine resistant strains of *Plasmodium falciparum* (Kovendan *et al*., 2012_a). When methanolic seed extracts of the seeds of *Carica papaya* was administered to *Plasmodium berghei* infected mice prophylactically at doses of 200, 100 and 50 gave prevention of 63.85, 61.12 and 48.08% prevention of malaria; however at the same doses given curatively the extracts failed to suppress parasitemia with mean survival of 6-8 days compared to 27.2 days of chloroquine treated rats (Amazu *et al*., 2009). Carpaine isolated from the alkaloid fraction of the leaf of *Carica papaya* showed highly active anti plasmodial activity in-vitro but could not be substantiated in-vivo (Juliante *et al*., 2014).

2.7.1.4 Anti-inflammatory effects

Ethanolic extract of *Carica papaya* leaves has shown to have anti-inflammatory activity against paw oedema, cotton pellet granuloma and formaldehyde induced arthritis model animals (Owoyele *et al*., 2008) and when the extract was tested on rats orogastrically dosed with *Staphylococcus aureas* and *Salmonella typhi* it was shown to also have anti-inflammatory effects (Oladunmoye *et al*., 2007). Mature leaf concentrate of the plant significantly inhibited careegenan induced rat paw oedema and impaired in vivo vascular permeability in mice by 82% while inducing maximum membrane stability activity of rats' red blood cells suggestive of anti-inflammatory activity (Gammulle *et al*., 2012). The aqueous extract of the seeds of *Carica papaya* significantly reduced pedal oedema by 83.5% compared to control and decreased formation of granulation tissue by 52.55% (Zubair and Ramabhimalah, 2012). Treatment of aspirin induced ulceration with aqueous extract of the fuits reduced ulcer index, lipid peroxide and alkaline phosphatase activity in rats and maintained catalase in gastric mucosa (Olagundudu *et al*., 2007). The intraperitonial administration of the methanolic extract of the seed of the plant has also been shown to have anti-inflammatory effect after the induction of paw inflammation using egg albumin (Amazu *et al*., 2010). Aqueous seed extract of the plant at 50 and 100mg/kg protected rat gastric mucosa against ethanol induced gastric ulcer by reducing gastric juice volume and acidity. Pretreatment with the extract exhibited anti-ulcerogenic effect on indomethacin induced peptic ulcer (Das *et al.,* 2016).

2.7.1.5 Anti-coagulant effects

Anti-coagulant factor from the latex of *Carica papaya* has shown that it inhibits the formation of a perfect fibrin clot from plasma, even the presence of active thrombin (Pillai *et al*., 1955). The latex from the unripe fruits significantly increased clotting time of whole blood and showed a dose dependent increase in the bleeding time with insignificant difference between the test groups and the heparin and aspirin treated control groups (Asare *et al*., 2015). The study was conducted to investigate the platelet increasing property of *Carica papaya* leaf juice (CPLJ) in patients with dengue fever (DF). An open labeled randomized controlled trial was carried out on 228 patients with DF and dengue hemorrhagic fever. The plant increases platelet count in test subjects versus control groups (Soobitha *et al*., 2013).

2.7.1.6 Effect on smooth muscle

The ethanolic extract of the seed of *Carica papaya* at 0.1-6.4mg/ml have been found to have a concentration based inhibition of jejunal contractions; thus the seed has the ability of weakening isolated rabbit jejunum (Krishna *et al*., 2008).

2.7.1.7 Anti-helminthic and anti-amoebic activity

The powder of the aqueous extracts of the seeds of *Carica papaya* has shown to significantly reduce the concentration of *Hetrakis gallinarum*, *Ascaridia galli* and *Trichostrongylus tenius* in commercial layers (Ameen *et al.,* 2012). Crude and aqueous seed extracts of *Carica papaya* was administered to West African Dwarf Goats and the extract treated groups showed significant increase in the lymphocyte count, packed cell

volume, red blood cell and hemoglobin concentration with significant decrease in eosinophil counts with reduction in fecal egg count of the helmith comparable to a standard drug thiabendazole (Effendry *et al*., 2014). Single dose of the same seed extract given to mice at a dose of 0.1ml/mice/day reduced parasite appearance of *Entamoeba histolytica* in feces completely clearing it by day 8 whereby metronidazole, a standard drug completed clear out by day 10 (Mohammed *et al*., 2014). Air dried *Carica papaya* seeds given to children with intestinal parasitosis reduced it by 76.7% (Okeniyi *et al.*, 2007). When the ethanol leaf extract was tested on the worms of *Ascaris suum* the ED_{50} was determined to be 12.5mg/ml (Wassawa and Olila, 2006). Antihelmitic activity has been ascribed to an alkaloid, carpaine and benzylthiourea and benzylisothiocyanate (Krishna *et al*., 2008).

2.7.1.8 Free radical scavenging

Different parts of the *Carica papaya* plant (fruits, leaf and seed) were tested for their antioxidant and free radical scavenging activity and it was determined that the young leaf had the highest free radical scavenging and antioxidant activity followed by the unripe fruit, ripe fruit and lastly seeds (Maisarah *et al*., 2013). The leaf of the plant had significant antioxidant and free radical scavenging activity in a concentration dependent manner with IC_{50} 7.33mg/ml and 1.58mg/ml for inhibition of erythrocyte haemolysis and lipid peroxidation respectively (Okoko and Ere, 2012). Aqueous leaf extract also showed a dose dependent free radical scavenging activity against DPPH, ABTS, nitric oxide and superoxide but only moderate activity against hydroxyl radical and lipid peroxidation potential in a study carried out by Srikanth *et al.* (2010). A different study on the effect of different fractions of the seeds (ethanol, petroleum ether, ethylacetate, n-butanol and water)

showed the ethylacetate fraction having the highest DPPH and hydroxy free radical scavenging activity greater than that of ascorbic acid and sodium benzoate (Zhou *et al*., 2011). Yeast fermentation of *Carica papaya* has been seen to scavenge 80% hydroxyl radicals. The oral administration of the preparation for four weeks decreased elevated lipid peroxide levels. It also increased superoxide dismutase activity in the cortex and hippocampus of experimental rats (Irnao *et al*., 1998; Srikanth *et al*., 2010). Aqueous extract of the fruit *Carica papaya* was able to counteract the effects of lipid peroxidation and acylamide induced oxidative stress in rats by increasing gluthatione levels and increasing the activity of catalase and superoxide dimutase (Sadek, 2012).

2.7.1.9 Hepatoprotective activity

The ethanol and aqueous extract of the fruit of the plant has shown to have remarkable hepatoprotective ability against CCL_4 induced hepatotoxicity (Rajkapoor *et al.,* 2002; Krisha *et al*., 2008). The aqueous leaf extract was able to decrease the markers of liver damage in rats with maximal activity observed at a concentration of 400mg/kg. Dried fruits of *Carica papaya* also significantly ($p<0.05$) reduced the levels of ALT and AST in CCl_4 induced liver damage in rats comparable with those administered vitamin E, however alkaline phosphatase reduction was higher in the extract treated group than in the vitamin E treated group (Sadeque *et al*., 2012). The stalk of the plant extracted in methanol also showed maximal activity at 100mg/kg when simultaneously administered with CCl_4 (Minan and Bamisaye, 2013). Aqueous extract of the leaves was shown to reduce markers of liver damage and also increase levels of SOD, GSH and total protein (Pandit *et al*., 2013).

2.7.1.10 Anti-sickling effect

Pre treatment with methanolic leaf extracts of CP were tested for anti-sickling properties. It was observed that reduced hemolysis and protected erythrocyte membrane integrity under osmotic stress conditions (Imaga *et al*., 2009). Butanol and ethylacetate fractions of the leaf of the plant showed anti-sickling activity at 10mg/ml and 5mg/ml respectively having sickling of 0-5% after 60mins when compared to 80% of the untreated control group (Imaga and Adepoju, 2010). Another study by Naiho *et al.* (2015) showed the leaf having a dose dependent significant ($p<0.05$) reduction in its ability to reverse the sickling of the tested cells but also showed significant ($p<0.05$) reduction in osmotic fragility when compared with controls. The fruit pulp in distilled water, methanol and chloroform using sodium metabisulphite sickled red blood cells showed 87% inhibitory and 74% reversal, 64% inhibition and 55% reversal respectively with the chloroform extract being inactive (Ogunyemi *et al*., 2008). Caricapinoside (8(2-0-β-D-4, 5-anhydroglucitoyl 1 → 2 glucopyranosyl carbonyl) di benzo 1, 4 dioxine 2, carboxylic acid) from the methanol extract of the unripe fruit of *Carica papaya* was also shown to have significant anti-sickling activity (Odunola *et al*., 2012).

2.7.1.11 Anti-diabetic effect

A study on the aqueous extract of the leaves at 0.75 and 1.5g/100ml significantly decreased blood glucose levels in diabetic rats. It also decreased cholesterol, triacylglycerol and amino transferase blood levels with prevention of hepatocyte disruption (Rojop *et al*., 2012). Crude extract of the seeds of *Carica papaya* at doses of 100 and 200mg/kg decreased blood glucose in a dose dependent manner (Venkateshwarlu *et al*., 2013).The

aqueous extract of *C. papaya* leaves significantly reduced the plasma blood glucose level and serum lipid profile in diabetic rats. The ethanolic extract of *C. papaya* leaves demonstrated significant reduction in blood glucose level and regeneration of the beta cells of pancreas in diabetic mice. Aqueous extract of unripe papaya fruit significantly inhibited the key enzymes -amylase and glucosidase involved in type 2 diabetes and also inhibited the lipid peroxidation in rat pancreatic cells studied *in vitro* (Natarajan, 2014). Ethanol leaf extract in alloxan induced diabetic rats significantly reduced blood glucose levels, total cholesterol, triglyceride and increased HDL (Adenowo *et al.,* 2014). Another study on the aqueous leaf and seed extracts administered to alloxan induced diabetic rats orally for 28 days. Both had hypoglycemic, hepatoprotective and nephroprotective effects with the seed being more effective than the leave (Ojo *et al.*, 2015). Unripe pulp of the plant elicited significant ($p<0.05$) reduction in blood glucose and lipid profile except HDL which increased (Ezekwe *et al.*, 2014). The ethanol leaf extract was also shown to have pancreatic islets protective characteristic as shown in the study by Miranda-Osorio *et al*. (2016) that the extract preserved the number and morphology of the pancreatic islets, improved basal insulin secretion and protected the cultured cells from adverse effects of streptozotocin.

2.7.2 Larvicidal activity of *Carica papaya*

Crude extracts of the plant have been tested such as crude methanol extract of the leaf of *C.papaya* showed highest activity against the first and fourth instar of *Aedes aegypti* with LC_{50} values of 51.76ppm and 82.18ppm and pupae was 440.65ppm (Kovendan *et al.,* 2012_b). In 2006, Okolie tested the crude aqueous extract of the leaves against anopheles and culex species and reported 100% morality at doses of 0.06mg/ml and 0.10mg/ml

respectively. In Indonesia, 70% crude ethanol extract of leaves and seeds were tested against the larvae of *Anopheles farauti*, *koliensis*, *subpictus* and *punctulatus* with LC_{50} and LC_{90} values for leaves and seeds being 422.311 ppm, 1399.577 ppm and 21.983 ppm and 137.862 ppm respectively (Sesanti *et al*., 2014). A comparative study was carried out between aqueous and ethanol extract of seeds of *Carica papaya* against the eggs and larvae of *Heligmosomoides bakeri* and ethanol extract was found to cause 96% mortality of the larvae and the crude aqueous extract reduced embryonation by 92% (Wabo *et al*., 2011). A presentation at the 3rd International congress on Global Warming in Bharathiar University, Colmbatore, India on the larvicidal and pupicidal activity of crude aqueous extract of *Carica papaya* leaf and seed extract against *Aedes aegypti* found that the extracts inhibited larval growth (Noortheen *et al*., 2013). Infra red analysis of the seeds of *Carica papaya* against the larvae of *Culex quinquefasciatus* and *Anopheles stephensi* showed the presence of aliphatic amide that may be responsible for the larvicidal activity (Rawani *et al*., 2012). Acetone extract of *Carica papaya* showed highest mortality when compared to methanol, ethanol, ethylacetate and chloroform extract against *Culex quinquefasciatus*. Mortality observed was 61.6% at 24 hrs at concentration of 500 ppm. LC_{50} and LC_{90} values were 80.56 and 380.67 ppm and 60.89 and 150.75 ppm at 24 and 48 hours respectively (Ravichandran *et al*., 2014). The effects of extracts of *Carica papaya* has not been tested on *Aedes vittatus* which is ubiquitous in Zaria, Kaduna State. Different parts of the plant have shown larvicidal activity in different solvents. This is indicative of the fact that the activity of this plant is not restricted to a single or particular part. Also the fact that different solvent show varying degrees of activity suggest that both polar and non polar constituents within the plant may affect larval mortality via different mechanisms. These

mechanisms have not been evaluated. A lot of emphasis has been placed on the seed of the plant but even at that very little is known of the active principles responsible for this activity. On the other hand nothing is known of the active principles that may exist in the leaf, stem or root of the plant that may be responsible for larvicidal activity (if it exists) or mechanisms of action by these principles. Though several extracts and compounds from different plant families have been evaluated to show new and promising larvicides, very few plant products have been developed for controlling mosquitoes (Magadula *et al.*, 2009).

2.8 *Dacryodes edulis*

Taxonomy

Kingdom - Plantae

Order -Sapiridales

Family -Burseraceae

Genus -Dacryodes

Species -*D. edulis*

Dacryodes edulis is an evergreen tree attaining the height of 18-40m in the forest and not exceeding 12m in plantations. The upper surfaces of the leaves are glossy and they are arranged in a large inflorescence. The fruit is an ellipsoidal drupe and the skin is dark blue or violet. The trees are male, female or hermaphrodite. Flowering takes place from January – April followed by the fruiting season between May- October. There is a minor fruiting season between November and March (Orwa *et al.*, 2009). The pulp of the fruit contains

considerable about of carbohydrates, lipids, proteins, fibre and mineral salts especially calcium, magnesium, potassium and sodium (Fotso *et al*., 2014). It is known as safou in French, Ube in Ibo, Elemi in Yoruba, Eben in Efik and Orumu in Benin (Onuegba *et al*., 2011). *Dacryodes edulis* is an underutilized tree crop. It is consumed in Nigeria raw, roasted or boiled in hot water and is eaten alone or used to garnish fresh maize. It is sometimes used to butter bread. Fruits are a source of amino acids or triglyceride with a saturated fat content of 50.85% and unsaturated fatty acid content of 49.14%. It is found in Nigeria, Cameroun, Sierra Leone, Uganda, Malaysia, Liberia and Zaire (Ibanga and Okon, 2009). In ethnomedicine the bark is used to treat wounds, leprosy and tonsillitis (Nwokonkwo, 2014). The oil is used to treat various skin diseases and applied topically to relieve pains (Okwu and Nnamdi, 2008). The vapour of the leaf decoctions is used to treat fever. The leaves are made into plaster to treat snake bite in South-West Cameroon (Ajibesin, 2011). Scientifically the oil has been shown to have cardiovascular activity by reducing LDL cholesterol of rats, antimicrobial and antioxidant activity against *Staphylococcus aureus, Bacillus cereus* and *Escherichia coli.* The aqueous and ethanolic extracts of the leaves have also shown anti-sickle cell anaemia potential by normalizing the SS blood erythrocytes validating the use in traditional medicine. Oladimeji *et al.* (2012) reported the larvicidal activity of the crude ethanol extract of the stem at 10% w/v resulting in mortality of 100% at 24 hours against *Anopheles gambiae*. Leaves, stems and stem backs are used in ethnobotany in the treatment of otitis, ameobic dysentery, leprosy, anaemia and yellow fever (Fotso, *et al* 2014). The wood has general use in carpentry and tools. The stem exudes a resin that is sometimes used as glue (Omonihinmin, 2012). Its bark has long been used to cicatrize wounds and for the treatment of leprosy, spitting

blood, debility, stiffness, tonsillitis and skin diseases. The leaves are often crushed and the juice is used for generalized skin diseases (Ogunmoyole *et al*., 2012). The fruit pulp yields cooking oil. The fruit is eaten raw, boiled, roasted in hot ashes, cooked in salt water to form a kind of butter and with cocoyam, rice and bread. It is also used in a local vegetable soup in Yoruba land. The seeds serve as food for local house hold ruminants like sheep and goats (Isiuku *et al.,* 2008). Ethnopharmacological uses include the use of the resin mixed with palmoil as treatment of ectoparasite infection and other skin diseases. The leaves or bark are boiled in papwater and given to children to treat retarded growth and epilepsy. The roots are boiled and used for the treatment of beri-beri and rickets. Boiled leaves and roots are added to lemon grass and used to manage hypertension (Omonhinmin, 2012).

2.8.1 Research based medicinal uses of *Dacryodes edulis*

2.8.1.1 Antimicrobial activity

The essential oils of the plant resin were investigated for antimicrobial and antioxidant activities. The essential oil showed more potent antibacterial effect against bacteria such as *Staphylococcus aureus*, *Bacillus cereus, Escherichia coli*, *Salmonella enteric* and *Proteus mirabilis* than antifungal effect against *Candida albicans* and this effect was found to be due to the presence and high content of terpinen-4-ol (19.8%) and α-pinene (17.4) (Ajibesin, 2011). Two thousand gram per milliliter (2000g/ml) of the aqueous and ethanolic seed extracts showed antibacterial activity against *Bacillus subtilis, Staphylococcus aureus* and *Klebsiella* (Omogbai and Eneh, 2011; Nwokonkwo, 2014).

Figure 2.2: Image of *Dacroydes edulis* (African pear)

Source: Ondo-Azi *et al*., 2013.

2.8.1.2 Antioxidant activity

In Cameroon, the extracts of 42 medicinal plants used for anaemia, diabetes, AIDS, malaria and obesity were investigated for phytochemical substances and antioxidant properties. The leaves of *Dacryodes edulis* elicited very high antioxidant effect when analyzed against three assay methods: Folin (Folin Ciocalteu Reagent), FRAP (Ferric Reducing Antioxidant Power) and DPPH (1, 1-diphenyl-2-picrylhydrazyl) (Ajibesin, 2011). Ethanol extracts of *D. edulis* possess antioxidant phytochemicals that showed significant effects on the MDA levels, GSH and CAT activities in blood and liver tissues of rats. The study suggests that *D. edulis* and *F. exasperata* are effective in bringing about restorative activity against CCl_4 induced oxidative stress and tissues (blood and liver) damages in rats (Omonihinmin and Agbara, 2013).

2.8.1.3 Cardiovascular activity:

Dacryodes edulis oil was reported to decrease the LDL cholesterol level in serum of rats (Leudeu *et al*., 2006). Health related functions of dietary plants such as *D. edulis* was reported to include immunostimulation and nervous system action (Ajibesin, 2011).

2.8.1.4 Antidrepanocytary activity (anti-sickle cell anemia):

Among the 13 Congolese plants examined for antidrepanocytary activity, the aqueous and ethanol extracts of *D. edulis* leaves were discovered to normalize the SS blood erythrocytes, following the deoxygenation of haemoglobin in anaerobic condition, thus validating their use in traditional medicine (Mpiano *et al*., 2007).

2.8.2 Larvicidal activity of *Dacryodes edulis*

Larvicidal activity of the crude leaf extract of *Dacryodes edulis* against *Anopheles gambiae* was evaluated by Oladimeji *et al.* (2012). It was determined that 5%w/v resulted in 50% and 70% larvicidal activity while at a concentration of 10% w/v resulted in a 90% and 100% larvicidal activity at 12hrs and 24hrs respectively. Literature report that the plant contains several different classes of secondary metabolites. These metabolites are responsible for multiplicity of biological actions including larvicidal activity in many different plants. Albeit these, very little research has been carried out on the larvicidal activity of different parts of the plant in different solvents. The research that was conducted on this plant involved the evaluation of the crude extract. The limited research information available on the larvicidal activity of the plant does not go further to suggest the mechanism of action or the metabolites that may be responsible for the activity and no attempt has been made to isolate and characterize the principles responsible for this activity. Different solvents ought to be used to determine in which region (polar or non polar) the larvicidal activity resides in the different parts of the plant. The phytochemicals that may be responsible for the activity must be identified. The active principles and the mode/mechanism of action should be ascertained.

2.9 Mosquito Management Techniques

One of the ways to control mosquito transmitted diseases involve the control of the vectors for the interruption of disease transmission (Singh *et al*., 2006). It is believed that sleeping under insecticide treated nets can reduce malaria related deaths in children by up to 20%

and help protect populations in filariasis endemic areas from infection. Malaria management and prevention initiatives involve four scientifically proven interventions.

I. Support of mosquito treated insecticide nets (ITN'S)

II. Indoor residual spraying (IRS)

III. Intermittent preventive treatment for pregnant women (IPT)

IV. Diagnosis and treatment (D&T)

2.9.1 Insecticide treated nets

This represents a powerful means for controlling malaria in Africa because the mosquito vectors feed primarily indoors at night (Nicodem *et al*., 2010). This focuses on the distribution and use of Long Lasting Insecticidal Nets (LLINS), including evidence based health communication programs on the mode of malaria transmission and the importance of sleeping under ITN's. UNICEF estimates that sleeping under insecticide treated nets can reduce the overall child mortality by 20% (Govella *et al*., 2010). In Thailand and Malaysia, pyrehtroids are used for the treatment of bed nets for the vector control of *Culex quiquefasciatus* Say (Wan-Norafikah *et al*., 2013).

2.9.2 Indoor residual spraying

This involves the spraying of insecticide on the indoor walls of homes in malaria affected areas. This is based on the fact that after feeding many malaria species rest on the nearby wall while digesting the blood meal and thus the pre-spraying of those walls kill the mosquitoes before they can bite another victim reducing transmission. The insecticides used for IRS include DDT and though DDT use has been banned in most developed

countries due to the hazards associated with its use, it is still used for IRS in resource poor countries due to its cost and persistence (Ratovonjato *et al*., 2014). Pyrethroids such as deltametrin and permethrin are also used for IRS (Wan-Norafikah *et al*, 2013). Despite all the negativity, chemical insecticides are still essential for mosquito control in malaria endemic countries, especially in sub-saharan Africa (Bird, 2017).

2.9.3 Intermittent prevention and treatment

As the scourge of malaria continues, special considerations regarding the management of the infection in the most vulnerable groups are needed to achieve maximum safety and efficacy of control strategies (Gonzalez *et al*., 2014). Intermittent preventive treatment for pregnant women is an effective method in reducing the effects of malaria in both pregnant women and the unborn child by giving at least two doses of sulphadoxine-pyrimethamine. Owing to not yet well established physiological reasons, pregnant women are more susceptible to the effects of malaria infection with increased associated morbidity and mortality both in the mother and newborn (Rogerson *et al.,* 2007).

2.9.4 Diagnosis and treatment

Prompt parasitological confirmation by microscopy or Rapid Diagnostic Test (RDT) is recommended for all patients suspected of being infected with malaria before treatment commences. The World Health Organization recommends that regular efficacy monitoring should be undertaken by all malaria endemic countries that have deployed artemisinin combination therapy (ACT). Although ACT is still efficacious for treatment of uncomplicated malaria, artemisinin resistance has been reported in South East Asia

suggesting that surveillance needs to be intensified in all malaria endemic countries (Shayo *et al.,* 2014).

2.10 Malaria, Filariasis, Dengue Diagnosis and Treatment

Early diagnosis of the disease prevents death and contributes to reducing transmission. The introduction of artemisinin-based combination therapy (ACT) has improved malaria case management substantially. However, development and spread of ACT resistance may have drastic consequences for the recent malaria control achievements. For this reason it has become increasingly important to change from symptom-based presumptive treatment to parasitological confirmation of malaria infection before initiation of anti-malarial treatment. The use of parasite-based diagnosis will allow better targeting of anti-malarial drugs, and also provide an opportunity for other causes of fever to be identified and appropriately treated. Therefore, WHO now recommends that anti-malarial treatment be confined to laboratory confirmed cases only (Mubi *et al*., 2013). Several other drugs and combination of drugs are also being used in the treatment of malaria. Chloroquine may be used when the parasite is still sensitive. The most sensitive drug for *Plasmodium falciparum* is the use of the artemisinins in combination with other antimalarials (known as artemisinis combination therapy ACT). The additional antimalarials include amodiaquine, lumefantrine, mefloquine or suphadoxime/pyrimethamine. Another recommended combination is artemisinin- piperaquine (Price and Douglas, 2009).

The recommended regimen for treatment of filariasis is through mass drug administration in a single dose of albendazole and ivermectin or diethylcarbamazine citrate given together (Sabesan *et al*., 2010). As regards the treatment of Dengue, there is no specific drug used

because it is caused by a virus. The treatment involves supportive measures and judicious fluid therapy (Senaka *et al*., 2012). In dengue, due to antibody mediated enhancement of disease severity, the use of vaccines to address the four antigenically different serotypes of the virus poses a problem (Rodriguez-Roche and Gould, 2013). There are no approved antiviral therapeutic agents to treat individuals presenting with symptoms of Dengue and Dengue hemorrhagic fever. The absence of validated animal models that faithfully reflects the symptoms of (DHF) observed in patients is a major challenge for the development of useful antivirals in DHF (Rodriguez-Roche and Gould, 2013).

Counterfeit drugs have been widely discovered in parts of Asia such as Cambodia and Indonesia. It is believed that up to one third of anti-malarial drugs in diseases endemic regions of sun-Saharan Africa are fake and are responsible for preventable 700,000 deaths every year (Karunamoorthi, 2014).

2.11 Drug Resistance

Drug resistance is the reduction in the effectiveness of a drug such as an antimicrobial, antihelmintic or an antineoplastic in curing a disease or condition. The immune system of an organism in broad terms is a drug delivery system. Resistance of the parasite to antimalarial medicines is a recurring problem. Resistance of *Plasmodium falciparum* to previous generations of medicines, such as chloroquine and sulpahdoxine-pyrimethamine (SP) was reported in the 1980's. Antimalarial drugs target the erythrocyte stage of the disease (Cui *et al.,* 2015). Resistance to artemisinins was reported in 2009 at the Cambodia-Thailand border. It is believed that resistance may be partially due to the use of artemisinins alone. The World Health Organization currently recommends the use of

combination therapy which involves the use of ACT. ACT consists of a potent artemisinin component and a longer acting partner drug. The ACT's recommend by WHO are arthemeter and lumefantrine, artesunate and amodiaquine, artesunate and metfloquine, dihydroarteminisin and piperaquine, artesunate and pyronaridine, artesunate/sulphadoxine pyrimethamine.

Patients tend to discontinue the use of the therapy prematurely following the rapid disappearance of symptoms; this results in incomplete clearance of parasites from the blood stream of the patient and these parasites may be passed on to another mosquito and transmitted to another person. If this resistance spreads to other geographical areas, the public health consequences could be dire as no alternative medicines currently exist. Malarial drug resistance is mediated by the rate at which de novo mutations conferring resistance appear and are selected through drug use within an individual and secondly by the spread of the resistant alleles to other individuals (Klein, 2013). Heritable drug resistance is enabled through a number of mechanisms including reductions in active or passive uptake of a drug, abrogation of the drug activity by conversion of the drug to another altered form, increased expression of the drug target, or a decrease in the ability of the inhibiting agent to bind due to alterations in the enzyme target (Cowman, 1998). Though drug resistance due to vector control programs that utilize mass drug administration is well known, factors that have mitigated against drug resistance in lymphatic filariasis include the combined use of drugs (albendazole, ivemectin and diethylcarbamazine) with different modes of action and the long and complex life cycle of the filariae (McCarthy, 2005). As no approved drugs exist for the treatment of dengue and yellow fever thus resistance does not currently exist.

Table 2.1: Classes of antimalarials

Class	Drug	Use
4 Aminoquinoline	Chloroquine	Treatment of non-falciparum malaria
	Amodiaquine	Partner drug for ACT
	Piperaquine	ACT partner drug with dihydroartemisinin as ACT
8-Aminoquinoline	Primaquine	Radical cure and terminal prophylaxis of *Plasmodium vivax* and *Plasmodium ovale*; gametocytocidal drug for *Plasmodium falciparum*
	Quinine	Treatment of *P. falciparum* and severe malaria
Arylamino alcohol	Mefloquine	Prophylaxis and partner drug for ACT for treatment of falciparum
	Lumefantrine	Combination with artemether as ACT
Sesquiterpene lactone endoperoxides	Artemether	ACT: combination with lumefantrine
	Artesunate	ACT; treatment of severe malaria
	Dihydroartemisinin	ACT: combination with piperaquine
Mannich base	Pyronaridine	Combination with artesunate as ACT
Antifolate	Pyrimethamine/sulfadoxine	Treatment of some chloroquine-resistant parasites; Combination with artesunate as ACT
Naphthoquinone /antifolate	Atovaquone/proguanil	Combination for prophylaxis and treatment of *P. falciparum* (Malarone)
Antibiotic	Doxycycline	Chemoprophylaxis; treatment of *P. falciparum*

ACT = artemisinin-based combination therapy. Source: Cui *et al*., 2015

2.12 Insecticide Resistance

Much of the successes in mosquito transmitted diseases involve vector control. This involves the use of pyrethroids and the continued use of oranophosphates (chlorpyrifos, temephos and fenthion) and insect growth regulators (diflubenzuron and methoprene) (Govidarajan and Glorintha, 2010). Synthetic organic chemical insecticides based intervention for the control of insect pests and insect vectors have disrupted natural biological control systems and led to outbreaks of insect species showing pesticide resistance such as those of malaria and filariasis (Singh *et al*., 2006). Pesticide resistance describes the decreased susceptibility of a pest population to pesticide that was previously effective at controlling the pest (Miyo, 2012). Pest species evolve pesticide resistance via natural selection; the most resistant specimens survive and pass on the genetic traits to their offspring. Among different categories of pests, insects are known to exhibit resistance at alarming rates. Worldwide more than 500 species of insects and related athropods are resistant to insecticides. Resistance may develop to only a single insecticide. However it is more common for insects that exhibit resistance to one insecticide to be resisitant to other insecticides with the same mode of action. The emergence of resistance of mosquitoes to insecticides threatens effective vector control (Ratovonjato *et al*., 2014). Response to insecticides can be categorized into physiological resistance and behavioural avoidance (Nkya *et al.*, 2014). Physiological resistance is the ability of an insect population to survive exposure to a concentration that would normally result in complete kill while behavioural avoidance is defined as the ability of an insect to move away from an insecticide treated area, often without lethal consequences (Chareonviriyaphap *et al.,* 2013). In Sub-Saharan Africa there are reports of widespread insecticide resistance and it is imperative that new

and alternative insecticides be developed. *Culex quinquefasciatus* collected from 19 countries in the United States of America have showm resistance to permethrin insecticides (Wan-Norafikah *et al.,* 2013). Detection of insecticide resistance should be an essential component of all national vector control efforts to ensure that the most effective vector control methods are being employed. The combination of phytochemicals that exist in plants with larvicidal activity make them better options in the prevention of insecticide resistance. The presence of larvicidal activity in different parts of the same plant and in different solvent are indicative of the fact that their activity is not limited to a single principle.

2.12.1 Factors influencing insecticide resistance

Insecticide resistance in field applications is multidimensional. The factors responsible for resistance can be classified as follows:

I. Genetic
II. Reproductive
III. Behavioral/Ecological
IV. Penetration resistance
V. Metabolic resistance
VI. Altered target site resistance (Nkya *et al*., 2013; Mohammed *et al*., 2015)

2.12.1.1 Genetics

Insects that carry genes that confer resistance to an insecticide or class of insecticides survive treatment and are selected to pass this resistance to future generations. These genes

may alter some components of the basic physiology. This is affected by the mutation rates and the relative fitness of the resistance genes (Gazave *et al.,* 2001).

2.12.1.2 Reproductive

This kind of resistance is determined by the number of generations per year. Survivors of pesticide use mate and pass on the resistance to their offspring, then subsequent generations with contain fewer susceptible individuals. Eventually the entire population may become resistant. The two major variables responsible for the rate at which resistant traits spread throughout a population are mechanism of inheritance such as if the genes dominant or recessive and severity of selection pressure ie the percentage of susceptible individuals survival in each generation and whether mortality occurs before or after they reproduce (Jensen *et al*., 2016).

2.12.1.3 Behavioral resistance

Resistant insects may detect or recognize a danger and avoid the toxin. This mechanism of resistance has been reported for several classes of insecticides, including organochlorines, organophosphates, carbamates and pyrethroids. Insects may simply stop feeding if they come across certain insecticides, or leave the area where spraying occurred (for instance, they may move to the underside of a sprayed leaf, move deeper in the crop canopy or fly away from the target area) (Chareonviriyaphap *et al*., 2013).

2.12.1.4 Penetration resistance

Resistant insects may absorb the toxin more slowly than susceptible insects. Penetration resistance occurs with reduction of insecticide uptake by reducing permeability of the

insect cuticle (Silva *et al*., 2012). This can protect insects from a wide range of insecticides. Penetration resistance is frequently present along with other forms of resistance, and reduced penetration intensifies the effects of those other mechanisms (Nkya *et al*., 2013).

2.12.1.5 Metabolic resistance

Resistant insects may detoxify or destroy the toxin faster than susceptible insects, or quickly rid their bodies of the toxic molecules. This may be through alteration in the levels of enzyme activities that degrade insecticides. Increased detoxification can be realized by increased activities of metabolic enzymes by mechanisms such as amplification, over expression and point mutations in coding sequences The rate of efficiency of these enzymes is usually higher in resistant insect strain. In addition to being more efficient, these enzyme systems also may have a broad spectrum of activity (i.e., they can degrade many different insecticides) (Miyo, 2012).

2.12.1.6 Altered target-site resistance

The site where the toxin usually binds in the insect becomes modified due to point mutation that results in insensitivity in genes that are target sites for insecticides (Sarwar and Salman, 2015).

2.13 Hazards and Environmental Pollution

Over the years the mosquito control programmes have been carried out by using synthetic insecticides. Repeated applications of synthetic insecticides for mosquito control has disrupted the natural biological control systems, killed non-target organisms and caused

harmful effects on humans (Al-Shaibani *et al.,* 2008) such as cardiopulmonary, neurological, skin disorders, fetal deformities, miscarriages and lowering the sperm count of applicators due to their broad spectrum of activity (Chinnamani *et al.,* 2016). Reducing pesticide use is one of high priority (Lechenet *et al*., 2014). Synthetic insecticides' non-biodegradable nature and their higher rate of biological magnification through the ecosystem are largely responsible for the increasing insecticide resistance on a global scale (Ghosh *et al*., 2012).

Pesticides persist in the environment; they leach into the soil and contaminate waterways. They are also found in nectar and pollen. Although vertebrates are less susceptible than arthropods to pesticides, consumption of dressed seeds offers a route of direct mortality in birds and mammals (Goulson, 2013). Exposure of the general population to insecticides occurs with eating food and drinking water contaminated with insecticide residues. Although pesticides have been developed to function with reasonable certainty and minimal risk to human health and the environment, published results are not always in agreement with the facts (Damalas and Eleftherohorinos, 2011). The Environmental Protection Agency has banned or placed restrictions on the use of many pesticides which were formerly used in mosquito control programme. There are fewer adulticides available today than there was 20 years ago. Manufacturers have withdrawn some insecticides due to the high cost of carrying out additional safety tests besides the use of adulticide to eradicate mosquito populations is not prudent as the adult stage occur along side human habitation as they can easily escape remedial measures (Chandra *et al*., 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

All chemicals were obtained commercially and were of analytical grade, these include ethanol, n-hexane, ethyl-acetate, chloroform, anhydrous chloroform, methanol, cyclo-hexane, methanol, acetone, Tween 80, phosphate buffer, BSA, DTNB, AChI, molisch's reagent, fehling's reagent, ferric chloride, acetic anhydride, sulphuric acid, sodium hydroxide.

3.1.2 Equipment and materials

Weighing balance (GF-2000), analytical balance, rotary evaporator BUCHI ROTAVAPOR R-205), water bath, Infrared spectrophotometer, GCMS, NMR, electric blender, tissue homogenizer, centrifuge, chromatographic column, microplate reader , TLC plates, filters, Whatman no 1 filter papers, measuring cylinder, pipette, disposable tips, drippers, strainer, disposable cups, Erlenmeyer flasks, beakers, reaction tubes, beakers, test-tubes, silica gel cromatofolios, developing tank with lid.

3.2 Methodology

3.2.1 Plant collection

The fresh seeds, leaves, stem and roots of *Carica papaya* were collected from Samaru, Zaria, Kaduna, Kaduna State while the fresh seeds, leaves, stem and roots of *Dacroydes edulis* were collected from Owerri, Imo State. They were identified and authenticated at the

herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, (A.B.U) Zaria. *Carica papaya* was identical to the samples on file with voucher no 12034 while *Dacroydes edulis* was identical to the samples on file with voucher no 1843. The specimens were air dried for 10 – 14 days and powdered separately in an electric blender.

3.2.2 Mosquito rearing

Larvae of *Aedes vittatus* was collected from Kufena Rock, Zaria, *Anopheles gambiae* was collected from Zaria Dam and *Culex quiquefasciatus* was collected around Ahmadu Bello University (A.B.U) water works, Zaria and identified at the Entomology Research Laboratory, Department of Biological Sciences, Ahmadu Bello University, Zaria. The larvae of *Aedes vittatus* and *Culex quinquefaciatus* were separately kept in specialized mosquito cages and allowed to develop into adults. The mosquitoes were maintained on 10% sugar solution. The males and females were allowed to mate and the females were fed on shaved and restrained rabbit for proper egg development. The larvae of *Anopheles gambiae* was grown and the late third and early fourth instar stages of it and *Aedes vittatus* and *Culex quinquefasciatus* were used for the rest of the study.

3.2.3 Extraction procedure

One hundred and twenty-five (125) grams of the powdered samples of the seed, leaf, stem and root were extracted by separately macerating them in cold 1 litre of 95% ethanol, distilled water and n-Hexane for 48 hours. Each solution was filtered using Whatman No1 filter paper. The filtrates were concentrated using a rotary evaporator and evaporated to

dryness in a water bath kept at 50^{0}C, the percentage yields were calculated. The samples were kept in clear sample containers and stored in the refrigerator at 4^{0}C until use.

Formulae for calculating percentage yield

$$\% \text{ extraction} = \frac{\text{weight of extract}}{\text{weight of plant}}$$

3.2.4 Stock and test sample preparation

One percent (1%) stock solution for each was obtained by weighing one (1) g of each sample and dissolving it in 1ml acetone and made up to 100ml with de-chlorinated water using Tween 80 as emulsifier. The stock solutions were kept in twenty-four (24) separate containers with aluminium foil over the mouth of the vial and screw capped.

Sample preparation

Stock solutions were prepared for the following

Ethanol, aqueous and hexane extract of *Carica papaya* seeds

Ethanol, aqueous and hexane extract of *Carica papaya* leaves

Ethanol, aqueous and hexane extract of *Carica papaya* stems

Ethanol, aqueous and hexane extract of *Carica papaya* roots

Ethanol, aqueous and hexane extract of *Dacroydes edulis* seeds

Ethanol, aqueous and hexane extract of *Dacroydes edulis* leaves

Ethanol, aqueous and hexane extract of *Dacroydes edulis* stems

Ethanol, aqueous and hexane extract of *Dacroydes edulis* roots

Experimental design

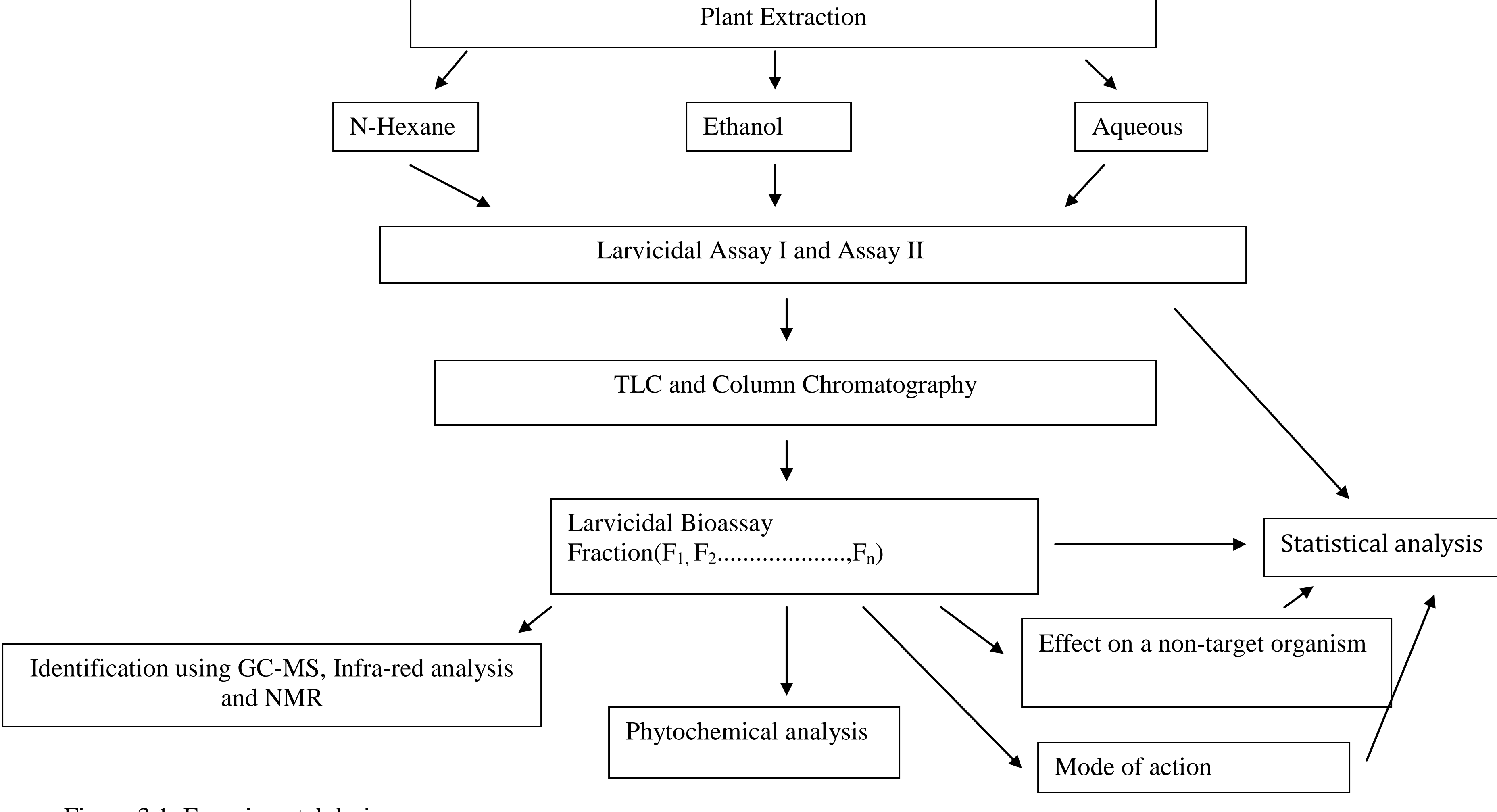

Figure 3.1: Experimental design

3.2.5 Larvicidal testing (Assay I)

Principle

The principle of the test is based on exposing mosquito larvae for a given time (usually 24-48 hours) in a container containing a known concentration of the test substance (larvicide) to determine the susceptibility of the larvae. This is determined by calculating the percentage of dead/moribund candidates in the total population.

Experimental design

Five hundred part per million (500ppm (50mg/dl) of the different extracts in 100ml of de-chlorinated water were placed in 250ml disposable test cups. Twenty-five larvae of late third instar and early fourth instar of each specie were transferred by means of a strainer into seperate disposable test cups. Small, unhealthy or damaged larvae were not used. There were twenty-four test groups and two control groups. Larvae were fed with crackers:yeast (3:1) (larvae food) (*Guidelines for larvicidal testing,* 2005) with modifications.

3.2.5.1 Evaluation of larvicidal activity

Group 1: Control

Consisted of 100ml de-chlorinated water with 1ml acetone, Tween 80, larvae and larvae food

Group 2: Control

Consisted of 3%v/v ethanol with larvae and larvae food

Group 3: Test groups

Test groups 3 - 26

Test groups comprised of aqueous, ethanol and hexane extracts 500ppm (50mg/dl) of the seeds, leaves, stems and roots of *Carica papaya* and *Dacryodes edulis* in 100ml of de-chlorinated water with the larvae and larvae food. All test groups were in triplicate.

The test containers were held at room temperature $25^{0}C$ – $28\ ^{0}C$ and in a photoperiod of 12h light by 12h dark (12L:12D).

To determine mortality, the larvae in the test containers were gently disturbed and made to go below the water surface by agitating the water with a sterile pipette. The living larvae that were able to swim to the surface were allowed to do so for 5 minutes following agitation. The larvae remaining or staying at the bottom of the recovery cups unable to swim to the surface were regarded as dead and recorded.

Larval mortality was recorded at 12, 24 and 48 hours.

Percentage mortality (PM) was determined using the equation

$$\%PM = \frac{\text{No of dead larvae}}{\text{Total larvae population}} \times 100$$

(Fred- Jaiyesimi and Anthony, 2011a)

Extracts of each plant part with mortality above 70% at 48 hours against at least two (2) of the larval species (PM≥70%) were selected for LC_{50} and LC_{90} determination.

3.2.6 Larvicidal testing (Assay II)

Experimental design

LC_{50} and LC_{90} Determination

To determine the LC_{50} and LC_{90} values of the different extracts with percentage mortality above 70% against at least two (2) larval species in assay I; concentrations of 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl), 100ppm (10mg/dl), 200ppm (20mg/dl) and 400ppm (40mg/dl) were prepared from the 1% stock solution. Six extracts were selected.

3.2.6.1 Evaluation of larvicidal activity

Late third and early fourth instar larvae of *Aedes vittatus*, *Anopheles gambiae* and *Culex quiquefasciatus* were used.

Group 1: Control

Consisted of 100ml of de-chlorinated water with addition 1ml acetone, Tween 80, larvae and larvae food

Group 2: Control

Consisted of 3% v/v ethanol with the larvae and larvae food

Test Groups (Group 3-44)

Group 3-9: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl), 100ppm (10mg/dl), 200ppm (20mg/dl) and 400ppm (40mg/dl) of ethanol extract of seed of *Carica papaya*

Group 10-16: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl), 100ppm (10mg/dl), 200ppm (20mg/dl) and 400ppm (40mg/dl) of hexane extract of seed of *Carica papaya*

Group 17-23: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl), 100ppm (10mg/dl), 200ppm (20mg/dl) and 400ppm (40mg/dl) of ethanol extract of leaf of *Carica papaya*

Group 24-30: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl), 100ppm (10mg/dl), 200ppm (20mg/dl) and 400ppm (40mg/dl) of hexane extract of leaf of *Carica papaya.*

Group 31-37: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl), 100ppm (10mg/dl), 200ppm (20mg/dl) and 400ppm (40mg/dl) of ethanol extract of seed of *Dacryodes edulis*

Group 38-44: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl), 100ppm (10mg/dl), 200ppm (20mg/dl) and 400ppm (40mg/dl) of hexane extract of leaf of *Dacryodes edulis*

All test concentrations were prepared in 100ml de-chlorinated water. Larval maintenance, test conditions and mortality were determined as in 'Assay I' above. Larval mortality, LC_{50} and LC_{90} values were recorded at and determined for 12, 24 and 48 hours. LC_{50} and LC_{90} values were determined using SPSS 20 software.

3.2.7 Larvicidal testing (Assay III)

Experimental design

The different fractions gotten from "Column Chroamtography I" of *Carica papaya* hexane leaf extract and *Dacryodes edulis* ethanol seed extract were subjected to larvicidal testing to determine in which fraction (F_x) larvicidal activity resides using LC_{50} values.

To determine the larvicidal activity of the fractions, concentrations of 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) were prepared from the fractions.

3.2.7.1 Evaluation of larvicidal activity

Group 1: Control

Consisted of 100ml of de-chlorinated water with addition 1ml acetone, Tween 80, larvae and larvae food

Group 2: Control

Consisted of 3% v/v ethanol with the larvae and larvae food

Test Groups 3-62

Extract 1 (*Carica papaya* hexane leaf extract)

Group 3-7: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 1

Group 8-12: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 2

Group 13-17: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 3

Group 18-22: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 4

Group 23-27: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 5

Group 28-32: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 6

Group 33-37: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 7

Group 38-42: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 8

Group 43-47: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 9

Group 48-52: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 10

Group 53-57: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 11

Group 58-62: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 12

Test Groups (63-97)

Extract 2 (*Dacryodes edulis* ethanol seed extract)

Group 63-67: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 1

Group 68-72: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 2

Group 73-77: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 3

Group 78-82: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 4

Group 83-87: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 5

Group 88-92: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 6

Group 93-97: The test groups comprised of larvae separately exposed to 6.25ppm (0.625mg/dl), 12.5ppm (1.25mg/dl), 25ppm (2.5mg/dl), 50ppm (5mg/dl) and 100ppm (10mg/dl) of Fraction 7

All test concentrations were prepared in 100ml de-chlorinated water and tested on the larvae of *Aedes vittatus* and *Culex quinquefasciatus*

Larval maintenance, test conditions and mortality were determined as in 'Assay I and II' above. Larval mortality was recorded at 12, 24 and 48 hours.

LC_{50} and LC_{90} values were determined for 12, 24 and 48 hours. Values were determined using SPSS 20 software.

3.2.8 Larvicidal testing (Assay IV)

Larvicidal activity of sub fractions from "Column chromatography II" of *Carica papaya* hexane leaf extract fraction 1 (CPHLE-f1) and *Dacryodes edulis* ethanol seed extract

fraction 1 (DEESE-f1) were determined using 12.5ppm (1.25mg/dl) and 25ppm (2.5mg/dl) concentrations against *Aedes vittatus*. Larval maintenance, test conditions and mortality were determined as in 'Assay I , II and III' above. Larval mortality was recorded at 12 and 24 hours.

LC_{50} value was determined for 12 and 24 hours. Values were determined using SPSS 20 software.

3.2.9 Larvicidal testing (Assay V)

Larvicidal activity of fractions 1 and 2 from the "Gel filteration" of sub-fraction 3 of *Carica papaya* hexane leaf extract fraction 1 (CPHLE-f1) was determined using 12.5ppm (1.25mg/dl) and 25ppm (2.5mg/dl) concentrations against *Aedes vittatus*. Larval maintenance, test conditions and mortality were determined as in 'Assay I , II and III' above. Larval mortality was recorded at 12 and 24 hours.

LC_{50} value was determined for 12 and 24 hours. Values were determined using SPSS 20 software.

3.2.10 Thin layer chromatography

The extracts with consistently low LC_{50} values against the larval species from "Larvicidal Assay II" were subjected to Thin Layer Chromatography (TLC) to determine their solvent systems as low LC_{50} values is an indication of the potentiality of the extract.

1. Hexane extract of the leaf of *Carica papaya*
2. Ethanol extract of the seeds of *Dacryodes edulis*

Principle

Thin layer chromatography is based on the principle that the partitioning of compounds/substances based on their adsorption to the stationary phase versus affinity for the mobile phase.

Method

Pre-coated TLC gel plates F_{254} were utilized. A line was drawn with a pencil approximately 0.5cm from the bottom. A micropipette was used to spot the sample on the line. Different solvent combinations were used to determine the best solvent system for the separation of the components of each extract. The plates were placed in the chamber, ensuring the solvent does not rise above the line at the bottom of the plate at the commencement of the experiment. The plates were removed before the solvent reached the top of the plate, the solvent line was then marked and the plate dried. Plates were sprayed with 10% sulphuric acid and gently heated in an oven.

1. Hexane extract of the leaves of *Carica papaya* (Hexane: Ethylacetate: Methanol) (8:2:1)
2. Ethanol extract of the seeds of *Dacryodes edulis* (Chloroform: Ethylacetate) (7:3)

3.2.11 Column chromatography I

The extracts selected from “Larvicidal Assay II” and subjected to Thin Layer Chromatography to determine their solvent systems were fractionated using column chromatography.

Principle: This separation technique is based on the principle that compounds are partitioned based on their properties of adsorption to the stationary phase (placed in a column) and elution using a mobile phase which is a solvent or combinationof solvents.

Method

The extracts were fractionated by column chromatography (CC). The column of length 65cm, size 15 was packed with 100g of activated silica from 60 to 200 mesh size; elution was performed with solvents determined from the TLC above.

Fifty millilitres (50mls) of the eluents were collected in beakers. The fractions from the column were subjected to thin layer chromatography. Fractions containing the components with same R_f values were combined. Flow rate was 50mls in 15 minutes.

R_f (retention factor) values of the separate components were calculated using the equation below:

$$R_f = \frac{\text{Distance travelled by the component}}{\text{Distance travelled by the solvent}}$$

Fractions were subjected to phytochemical analysis.

3.2.12 Column chromatography II

The fractions selected from "Larvicidal Assay III" were further fractionated by column chromatography (CC). The column of length 25cm, was packed with activated silica from 60 to 200 mesh size; elution was performed with hexane:ethylacetate 9:1. The sub-fractions were collected in 5ml's in beakers. See 3.2.11 above.

3.2.13 Phytochemical Analysis

The fraction(s) (F_x) from "Column Chromatography I" above were subjected to qualitative phytochemical screening using standard methods (Trease and Evans, 1989; Sofowora, 1993).

3.2.13.1 Test for carbohydrates

Principle: The test is based on the principle of the dehydration of carbohydrate by sulphuric acid or hydrochloric acid to produce an aldehyde which condenses with two molecules of phenol resulting in a red or purple coloured compound.

Method

General test – Molisch's test: To 2 mls of the stock solution, few drops of molisch's reagent was run down the inclined tube to form a lower layer without shaking the tube. A purple ring at the interphase of the liquids indicated the presence of carbohydrates.

3.2.13.2 Test for tannins

Principle

The test is based on the reaction between phenol and ferric chloride to produce a violet complex

Method

Ferric chloride test: To a little portion of the stock solution, distilled water was added in the ratio of 1:4 and few drops of 10% ferric chloride was then added. Absence of blue or green colouration indicated absence of tannins.

3.2.13.3 Test for saponins

Principle

This is based on the fact that saponin glycosides form colloidal solution in aqueous solution that foams upon agitation.

Method

Froth test*:* Ethanol (3 mls), and was added to the fractions and mixed with 10ml distilled water in a test tube. The tube was stoppered and shaken vigorously for about 5 minutes and allowed to stand for 30 minutes and observed for honey comb froth.

3.2.13.4 Test for terpenes and steroids

Principle

Is a test for unsaturated steroids. Reaction of acetic anhydride with terpenes in chloroform solution produces a characteristic green or blue green colour and a reddish colour at the interphase.

Method

The fractions were dissolved in 95% ethanol, filtered and the filtrate evaporated to dryness in a water bath. The residue was then dissolved in 10ml of anhydrous chloroform and filtered. The filtrate was divided into equal portions and the following tests were carried out.

Lieberman – Burchard test: The first portion of the chloroform extract from above was mixed with 1ml of acetic anhydride followed by addition of 1ml concentrated sulphuric acid down the side of the test tube to form a layer. A reddish colour at the junction of the two liquids and a green colour in the chloroform layer indicated the presence of terpenes.

Principle

The principle is based on the protonation of the hydroxyl group of sterols by concentrated sulphuric acid to form a colored conjugated diene.

Method

Salkwoski's test: The second portion of the chloroform solution was mixed with concentrated sulphuric acid carefully, so that it formed a lower layer. A reddish brown colour at interphase indicated the presence of steroidal rings (aglycone part of the cardiac glycoside).

3.2.13.5 Test for flavonoids

Principle

The deprotonation of the polyphenolic molecules in flavonoids turns the phenols to phenoxides, a yellow compound that decolorizes on the addition of hydrochloric acid.

Method

The fractions were detanned with acetone. The residue was then extracted in warm water after evaporating off the acetone on a water bath. The mixture was filtered while hot and the filtrate used for the tests.

Sodium hydroxide test: To an equal volume of the filtrate, 5ml of 10% sodium hydroxide was added. Yellow colouration indicated the presence of flavonoids. Addition of hydrochloric acid decolourized the sample.

3.2.13.6 Test for cardiac glycosides

Kedde's test

Principle

Is based on the ability of cardenolide (5 member lactone ring) to react with 3,5 dinitrobenzoic acid and sodium hydroxide to generate a characteristic purple violet colour.

Method

Chloroform was added to the fractions and filtered and one (1) ml of Kedde's reagent (2% 3,5 dinitrobenzoic acid in alcohol and 10% sodium hydroxide) was added to two (2) mls of the filterate. The presence of of purple violet colour indicated cardiac glycosides (-CH_2-group of lactone ring).

Keller-Killani test

Principle

This is based on the reaction of deoxy sugars of cardiac glycosides reacting with glacial acetic acid, ferric chloride and sulphuric acid to form a a blue coloured complex.

Method

Chloroform was added to the fractions and filtered. Two (2) mls of the glacial acetic acid (containing trace of ferric chloride) was added to two (2) mls of the fiterate . Concentrated sulphuric acid was added by the side of the testube. The formation of blue colour in the acetic acid layer showed presence of deoxy sugars.

3.2.13.7 Test for alkaloids

Principle

Alkaloids are precipitated from neutral and slightly acidic solution by Mayer's reagent (potassiomercuric iodide solution)

Method

Mayer's test: To an acidified solution of the fractions, few drops of Mayer's reagent was added. A white to yellow precipitate indicated the presence of alkaloids.

3.2.14 Mode of action

3.2.14.1 Adult emergence inhibition activity

Principle

Mosquitoes go through three aquatic phases before adult and taking flight. The transition between the four instars of the larval stage to pupae are regulated by concentrations of juvenile hormone and ecdysone. Larvicides that act as growth inhibitors act on principles such as but not restricted to mimicing juvenile hormone e.g methoprene. Monitoring emergence of mosquito metamorphosis from larvae to adult indicates a growth inhibitory activity.

Method

The effect of fraction(s) on the inhibition of adult emergence (IE) was determined by the use of the LC_{50} of each of the fractions on 1st – 4th instars of the larval species. Twenty five larvae were placed in capped one litre glass jar containing 100 ml of water in which the LC_{50} of the efficacious fraction (s) for each was mixed. Control groups were set up for each in a similar fashion. The number of emerged adults was recorded until adult emergence was completed in the control jars (Rajkumar and Jebanesan, 2005) modified.

Group 1-4: Control

Group 1: Twenty-five (25) 1st instar larvae of *Aedes vittatus* were placed in tests cups containing 100ml of de-chlorinated water

Group 2: Twenty-five (25) 2nd instar larvae of *Aedes vittatus* were placed in tests cups containing 100ml of de-chlorinated water

Group 3: Twenty-five (25) early 3rd instar larvae of *Aedes vittatus* were placed in tests cups containing 100ml of de-chlorinated water

Group 4: Twenty-five (25) late 3rd and early 4th instar larvae of *Aedes vittatus* were placed in tests cups containing 100ml of de-chlorinated water

Test groups 5-8

Group 5: 25 1st instar larvae of *Aedes vittatus* were placed in test cups containing 10ppm (1mg/dl) of *Carica papaya* hexane leaf extract – fraction 1 in 100ml of de-chlorinated water.

Group 6: 25 2nd instar larvae of *Aedes vittatus* were placed in test cups containing 10ppm (1mg/dl) of *Carica papaya* hexane leaf extract – fraction 1 in 100ml of de-chlorinated water.

Group 7: 25 early 3rd instar larvae of *Aedes vittatus* were placed in test cups containing 10ppm (1mg/dl) of *Carica papaya* hexane leaf extract – fraction 1 in 100ml of de-chlorinated water.

Group 8: 25 late 3rd and early 4th instar larvae of *Aedes vittatus* were placed in test cups containing 10ppm (1mg/dl) of *Carica papaya* hexane leaf extract – fraction 1 in 100ml of de-chlorinated water.

Group 9-12: Control

Group 9: Twenty-five (25) 1st instar larvae of *Culex quinquefasciatus* were placed in tests cups containing 100ml of de-chlorinated water

Group 10: Twenty-five (25) 2nd instar larvae of *Culex quinquefasciatus* were placed in tests cups containing 100ml of de-chlorinated water

Group 11: Twenty-five (25) early 3rd instar larvae of *Culex quinquefasciatus* were placed in tests cups containing 100ml of de-chlorinated water

Group 12: Twenty-five (25) late 3rd and early 4th instar larvae of *Culex quinquefasciatus* were placed in tests cups containing 100ml of de-chlorinated water

Test groups 13-16

Group 13: 25 1st instar larvae of *Culex quinquefasciatus* were placed in test cups containing 15ppm (1.5mg/dl) of *Carica papaya* hexane leaf extract – fraction 1 in 100ml of de-chlorinated water.

Group 14: 25 2nd instar larvae *Culex quinquefasciatus* were placed in test cups containing 15ppm (1.5mg/dl) of *Carica papaya* hexane leaf extract – fraction 1 in 100ml of de-chlorinated water.

Group 15: 25 early 3rd instar larvae of *Culex quinquefasciatus* were placed in test cups containing 15ppm (1.5mg/dl) of *Carica papaya* hexane leaf extract – fraction 1 in 100ml of de-chlorinated water.

Group 16: 25 late 3rd and early 4th instar larvae of *Culex quinquefasciatus* were placed in test cups containing 15ppm (1.5mg/dl) of *Carica papaya* hexane leaf extract – fraction 1 in 100ml of de-chlorinated water.

Group 17-20: Control

Group 17: Twenty-five (25) 1st instar larvae of *Aedes vittatus* were placed in tests cups containing 100ml of de-chlorinated water

Group 18: Twenty-five (25) 2nd instar larvae of *Aedes vittatus* were placed in tests cups containing 100ml of de-chlorinated water

Group 19: Twenty-five (25) early 3rd instar larvae of *Aedes vittatus* were placed in tests cups containing 100ml of de-chlorinated water

Group 20: Twenty-five (25) late 3rd and early 4th instar larvae of *Aedes vittatus* were placed in tests cups containing 100ml of de-chlorinated water

Test groups 21-24

Group 21: 25 1st instar larvae of *Aedes vittatus* were placed in test cups containing 10ppm (1mg/dl) of *Dacryodes edulis* ethanol seed extract – fraction 1 in 100ml de-chlorinated water.

Group 22: 25 2nd instar larvae of *Aedes vittatus* were placed in test cups containing 10ppm (1mg/dl) of *Dacryodes edulis* ethanol seed extract – fraction 1 in 100ml de-chlorinated water.

Group 23: 25 early 3rd instar larvae of *Aedes vittatus* were placed in test cups containing 10ppm (1mg/dl) of *Dacryodes edulis* ethanol seed extract – fraction 1 in 100ml de-chlorinated water.

Group 24: 25 late 3rd and early 4th instar larvae of *Aedes vittatus* were placed in test cups containing 10ppm (1mg/dl) of *Dacryodes edulis* ethanol seed extract – fraction 1 in 100ml de-chlorinated water.

Group 25-28: Control

Group 25: Twenty-five (25) 1st instar larvae of *Culex quinquefasciatus* were placed in tests cups containing 100ml of de-chlorinated water

Group 26: Twenty-five (25) 2nd instar larvae of *Culex quinquefasciatus* were placed in tests cups containing 100ml of de-chlorinated water

Group 27: Twenty-five (25) early 3rd instar larvae of *Culex quinquefasciatus* were placed in tests cups containing 100ml of de-chlorinated water

Group 28: Twenty-five (25) late 3rd and early 4th instar larvae of *Culex quinquefasciatus* were placed in tests cups containing 100ml of de-chlorinated water

Test groups 29-32

Group 29: 25 1st instar larvae of *Culex quinquefasciatus* were placed in test cups containing 10ppm (1mg/dl) of *Dacryodes edulis* ethanol seed extract – fraction 1 in 100ml de-chlorinated water.

Group 30: 25 2nd instar larvae *Culex quinquefasciatus* were placed in test cups containing 10ppm (1mg/dl) of *Dacryodes edulis* ethanol seed extract – fraction 1 in 100ml de-chlorinated water.

Group 31: 25 early 3rd instar larvae of *Culex quinquefasciatus* were placed in test cups containing 10ppm (1mg/dl) of *Dacryodes edulis* ethanol seed extract – fraction 1 in 100ml de-chlorinated water.

Group 32: 25 late 3rd and early 4th instar larvae of *Culex quinquefasciatus* were placed in test cups containing 10ppm (1mg/dl) of *Dacryodes edulis* ethanol seed extract – fraction 1 in 100ml de-chlorinated water.

All tests were carried out in triplicate. Larvae were fed with larvae food (crackers:yeast 3:1). Data was calculated using the formulae

$$IE\% = 100 - \left(\frac{T \times 100}{C}\right)$$

Where T= Percentage survival in treated C= Percentage survival in control

(*Guidelines for larvicidal testing*, 2005).

3.2.14.2 Acetylcholinesterase inhibition assay

Principle

Acetylcholinesterase catalyzes the hydrolysis of acetyl-thiocholine (sulfur analogs of their respective natural substrate, acetylcholine). Upon hydrolysis, these substrate analogs produce acetate and thiocholine. Thiocholine in the presence of the highly reactive dithiobisnitro-benzoate (DTNB) ion reacts to generate the yellow of 5-thio-2-nitrobenzoate anion. The yellow color, can be quantified by its absorbance at 405 nm (Ellman *et al.*, 1961).

Method

About 100 dead larvae were frozen at -20^{0}C, decapitated and the heads were homogenized in 5ml ice-cold 0.1M sodium phosphate buffer (pH 8.0), using a tissue homogenizer. The homogenate was centrifuged at 10,000 x g at 4^{0}C for 20 minutes. The supernatant was then filtered with a 0.22µm filter and used as the AChE preparation.

A microplate acetylcholinesterase (AChE) assay was carried out using a reaction mixture consisting of the 80µl of crude enzyme preparation, 10µlof 7.5mM DTNB in phosphate buffer (pH 7.0) and 6.25ppm, 12.5ppm, 25ppm, 50ppm and 100ppm of the fractions in 2.5% acetone. The reaction mixture was pre-incubated at 30^{0}C for 10 minutes and 10µl of 6.25mM acetylcholine iodide (ATChI) was then added to the mixture and allowed to stand for 10 minutes after which 10µl of 2% SDS in DTNB. The absorbance was then recorded at 405nm, using a microplate reader. Control was set up similar to test but without the addition of the test fractions (Dong and Young-Joon, 2013; Benabent *et al*., 2014)

Groups were set up as follows:

Group 1: Control

The control group contained 80µl crude enzyme with 10µl DTNB in phosphate buffer, 10µl ACTI and 10µl SDS in DTNB

Group 2-6: Test groups

Group 2: The test group comprised of 80µl crude enzyme with 10µl DTNB in phosphate buffer, 6.25ppm (.625mg/dl) of fraction 1 of *Carica papaya* hexane leaf extract, 10µl ACTI and 10µl SDS in DTNB

Group 3: The test group comprised of 80µl crude enzyme with 10µl DTNB in phosphate buffer, 12.5ppm (1.25mg/dl) of fraction 1 of *Carica papaya* hexane leaf extract, 10µl ACTI and 10µl SDS in DTNB.

Group 4: The test group comprised of 80µl crude enzyme with 10µl DTNB in phosphate buffer, 25ppm (2.5mg/dl) of fraction 1 of *Carica papaya* hexane leaf extract, 10µl ACTI and 10µl SDS in DTNB

Group 5: The test group comprised of 80µl crude enzyme with 10µl DTNB in phosphate buffer, 50ppm (5.0mg/dl) of fraction 1 of *Carica papaya* hexane leaf extract, 10µl ACTI and 10µl SDS in DTNB.

Group 6: The test group comprised of 80µl crude enzyme with 10µl DTNB in phosphate buffer, 100ppm (10mg/dl) of fraction 1 of *Carica papaya* hexane leaf extract, 10µl ACTI and 10µl SDS in DTNB.

Group 7: Control

The control group contained 80µl crude enzyme with 10µl DTNB in phosphate buffer, 10µl ACTI and 10µl SDS in DTNB

Group 8-12: Test groups

Group 8: The test group comprised of 80µl crude enzyme with 10µl DTNB in phosphate buffer, 6.25ppm (0.625mg/dl) of fraction 1 of *Dacryodes edulis* ethanol seed extract , 10µl ACTI and 10µl SDS in DTNB.

Group 9: The test group comprised of 80µl crude enzyme with 10µl DTNB in phosphate buffer, 12.5ppm (1.25mg/dl) of fraction 1 of *Dacryodes edulis* ethanol seed extract , 10µl ACTI and 10µl SDS in DTNB.

Group 10: The test group comprised of 80µl crude enzyme with 10µl DTNB in phosphate buffer, 25ppm (2.5mg/dl) of fraction 1 of *Dacryodes edulis* ethanol seed extract , 10µl ACTI and 10µl SDS in DTNB.

Group 11: The test group comprised of 80µl crude enzyme with 10µl DTNB in phosphate buffer, 50ppm (5mg/dl) of fraction 1 of *Dacryodes edulis* ethanol seed extract , 10µl ACTI and 10µl SDS in DTNB.

Group 12: The test group comprised of 80µl crude enzyme with 10µl DTNB in phosphate buffer, 100ppm (10mg/dl) of fraction 1 of *Dacryodes edulis* ethanol seed extract, 10µl ACTI and 10µl SDS in DTNB.

All tests were carried out in triplicate

$$\% \text{ inhibition} = \left(\frac{A_{con} - A_{sample}}{A_{con}} \right) X\ 100$$

Hematpoor *et al*., 2016

3.2.15 Effect on a non-target organism

Principle

The principle of this test is based on subjecting a non target organism of the same habitat to a sub lethal dose of the extract or fractions to determine if the mechanism of larvicidal action will also affect a non target organism of the same habitat.

Method

A test to determine the susceptibility of a selected non target organism to plant extracts involved the use of *Poecelia reticulata* (guppy-fish) as the non-target organism. The following stage test was conducted following the method of Promisiri *et al*. (2006).

Stage one assessment of toxicity

Guppy fish was subjected to the lowest concentration of the fraction (f1) *Carica papaya* hexane leaf extract and *Dacryodes edulis* ethanol seed extract (25ppm (2.5mg/dl)) that produced more than 50% larval mortality in the larvicidal test. Thirty guppy-fish were placed in containers containing 400 ml of the fractions seperately in dechlorinated water solution in three replicates. Controls, consisting of 30 fish in de-chlorinated tap water only, were studied at the same time. The number of dead fish was recorded first at 24-hours and then at 48-hours and the percentage mortality was calculated. All of these bioassay tests were conducted at room temperature without aeration or renewal of water.

Stage two assessment of toxicity

Toxicity was further assessed first at LC_{50} (*Carica papaya* leaf hexane (f1) 15ppm (1.5mg/dl)), (*Dacyodes edulis* seed ethanol (f1) 10ppm (1mg/dl)) value (point) and then at

LC_{90} (*Carica papaya* leaf hexane (f1) 326ppm (32.6mg/dl)), (*Dacyodes edulis* seed ethanol (f1) 58ppm (5.8mg/dl) value (point), where there was no significant toxic effect (had suffered no illness) to fish in stage one when compared to controls. Thirty guppy-fish were placed in containers containing 400 ml of fraction water solution in three replicates. Controls, consisting of 30 fish in de-chlorinated tap water, were studied at the same time. The number of dead fish was recorded first at 24-hours and then at 48-hours and the percentage mortality was calculated. All of these bioassay tests were conducted at room temperature without aeration or renewal of water.

3.2.16 Active principle determination

The partially purified fractions (f1) selected from "Larvicidal Assay III" were used for the determination of the active principle using IR, GCMS and NMR.

3.2.16.1 Infrared analysis

Principle

This is the identification of molecules based on the application of infra red radiation to those molecules. It results in vibration and rotation within the molecule which can be read and compared to a data base thereby identifying the functional groups of the molecule.

Method

The Infra red analysis of the fraction with the best larvicidal potentiality was carried out using Fourier Transform Infra-red spectroscopy (FTIR) Using FTIR Spectrometer, Agilent Technologies Cary 630 at Multiuser Science Research Laboratory, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

3.2.16.2 Gas chromatography-mass spectroscopy

Principle

Gas chromatography-mass spectroscopy is a separation technique used to analyze complex organic and biochemical mixtures. The GC-MS instrument consists of two main components. The gas chromatography portion separates different compounds in the sample into pulses of pure chemicals based on their volatility by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column. Spectra of compounds are collected as they exit a chromatographic column by the mass spectrometer, which identifies and quantifies the chemicals according their mass-to-charge ratio (m/z). These spectra can then be stored on the computer and analyzed (Hussain and Maqbool, 2014).

Method

The analysis was done at the Central Research and Diagnostic Laboratory, Tanke, Ilorin, Kwara State, Nigeria. The GC-MS was run on an Agilent 5975C Series GC/MSD. Column used was Agilent HP-5 5% Phenyl Methyl Siloxane with size 30m x 320µm x 0.25µm. The instrument was set to an initial temperature 35°C and maintained at this temperature for 5 minutes. At the end of this period the oven temperature rose to 150°C at a rate of an increase of 4°C and maintained for 2 minutes after which the oven temperature rose to 250°C at a rate of increase of 20°C/min and maintained for 5 minutes. Injection port temperature was 1.5ml/min. Ionization voltage was 70eV. The mass spectral scan range was set at 50-750 (m/z). Using computer searches on a NIST Ver.2 MS data library and comparing the spectrum obtained through GC-MS compounds present in the samples were identified.

3.2.17 Gel filteration

Sub-fraction 3 of fraction f1 of *Carica papaya* hexane leaf extract selected from "Larvicidal Assay IV" was further purified using gel filteration.

Principle

Gel filteration is a separation technique based on differences in molecular size of components of oranic solutions. The stationary phase is an inert compound usually sephadex and the mobile phase is an organic solvent.

Method

Sub-fraction 3 of fraction f1 of *Carica papaya* hexane leaf extract was placed in a column of 25cm length and 1cm diameter loaded with 10g of Sephadex LH-20. The sephadex was suspended in methanol and allowed to swell for 24 hours prior to use. The sample was dissolved in methanol and applied to the surface of the gel in the column. The sample was eluted with methanol and collected in 10 mls at a rate of 1 drop every 10 seconds. The sample yielded two distinct sub-fractions that were tested for larvicidal activity. Sub-fraction 2 was selected for NMR analysis.

3.2.18 Nuclear magnetic resonance

Principle

Samples are identified based on the principle that all nuclei of molecules have a spin based on the ratio of protons to neutrons. When an external electromagnetic force is applied the variation in that spin can be detected and recorded and used to elucidate the structure of the molecule.

Method

The NMR analysis of the fraction with the best larvicidal activity was carried out using Agilent Technologies 400 MHz NMR using chloroform as a solvent at Multiuser Science Research Laboratory, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

3.2.19 Statistical analysis

Mortality was corrected using Abbot (1925) formulae in test groups where deaths in negative control groups were between 5%-20%. Data from mortality analysis were analysed using ANOVA and Post-Hoc LSD (Fisher's Least Significant Difference). LC_{50} and LC_{90} was determined using probit analysis. Differences between means ± SD were considered significant at $p<0.05$.

Mortality may be corrected using Abbots formulae

$$\frac{\text{(mortality in test (\%)- mortality in control(\%))}}{\text{(100\%-mortality in control)}} \times 100 \qquad \text{(Abbot, 1925)}$$

CHAPTER FOUR

4.0 RESULTS

4.1 Determination of Percentage Yield of the Aqueous, Ethanol and N-Hexane Extracts of Seed, Leaf, Stem and Root of *Carica papaya* and *Dacryodes edulis* extracts

The plants were grouped based on their different parts and the solvent extracts of each part were compared based on their percentage yields. The hexane extract of the seeds of *Carica papaya* produced the highest yield with a value of 4.94%. The aqueous and ethanol extracts of the seeds of the plant gave yields of 0.75 and 2.94% respectively. The aqueous extract of the leaves of *Carica papaya* had the highest yield with the ethanol and hexane extracts yielding 2.09 and 1.31% respectively. The ethanol extract of the stem of *Carica papaya* had the highest yield with a value of 2.42% with the aqueous and hexane extracts having yields of 1.57 and 0.92%. The ethanol extract of the root of *Carica papaya* produced the highest yield of 3.39%. (Table 4.1).

The ethanol extracts of the seeds and leaves of *Dacryodes edulis* had the highest percentage yields with values of 2.42% and 1.45%. The aqueous extract of the stem had the highest yield with a value of 1.49%. The aqueous, ethanol and hexane extracts of the roots of *Dacryodes edulis* gave yields of 1.53, 1.49 and 1.09% respectively. (Table 4.1).

Table 4.1: Percentage Yield of the Aqueous, Ethanol and N-Hexane Extracts of Seed, Leaf, Stem and Root of *Carica papaya* and *Dacryodes edulis*.

	Extract	Yield (g)	% extract yield		Extract	Yield (g)	% extract yield
Group 1	CSA	0.94±0.17	0.75±0.14	Group 5	DSA	1.96±0.19	1.57±0.15
	CSE	3.68±0.57	2.94±0.46		DSE	3.03±0.17	2.42±0.13
	CSH	6.17±0.53	4.94±0.43		DSH	2.31±0.28	1.85±0.23
Group 2	CLA	3.03±0.27	2.42±0.22	Group 6	DLA	1.21±0.35	0.97±0.27
	CLE	2.87±0.52	2.09±0.78		DLE	1.81±0.34	1.45±0.28
	CLH	1.63±0.44	1.31±0.28		DLH	1.76±0.26	1.41±0.20
Group 3	CSTA	1.97±0.46	1.57±0.37[b]	Group 7	DSTA	1.87±0.31	1.49±0.25
	CSTE	3.03±0.37	2.42±0.30		DSTE	1.47±0.25	1.17±0.20
	CSTH	1.15±0.32	0.92± 0.26		DSTH	1.03±0.15	0.82±0.12
Group 4	CRA	3.21±027	2.57±0.21	Group 8	DRA	1.91±0.32	1.53±0.26
	CRE	4.24±0.66	3.39±0.52		DRE	1.86±0.19	1.49±0.15
	CRH	1.25±0.27	1.00±0.22		DRH	1.37±0.14	1.09±0.11

Key: C - *Carica papaya*, D - *Dacryodes edulis*, S - Seed, L - Leaf, ST - Stem, R - Root, A - Aqueous, E – Ethanol, H - Hexane

4.2 Larvicidal Testing

4.2.1 Assay I

The percentage mortality of the larvae of *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* when subjected to 500ppm of aqueous, ethanol and hexane extracts of the seeds, leaves, stems and roots of *Carica papaya* and *Dacryodes edulis* using 1ml of absolute ethanol as positive control. These are shown in Tables 4.2-4.9.

Table 4.2

Aqueous seed extract of *Carica papaya* against all three species did not show significant difference from negative control. The ethanol extract of the seeds of *Carica papaya* gave percentage mortality values of 73.34%, 76% and 100% against the larvae of *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* respectively at 48 hours. Maximal mortality against *Culex quinquefasciatus* was achieved at 24 hours. Hexane extract of the seeds of *Carica papaya* also showed larvicidal potential with an initial mortality of 70.6% against *Aedes vittatus*at 12 hours rising to 98.67% by 48 hours. Against the larvae of *Anopheles gambiae* and *Culex quinquefasciatus* maximal mortality was achieved at 48 hours with values of 92% and 72% respectively. The ethanol and hexane extracts caused significant mortality ($p < 0.05$) in all three species at 48 hours when compared with the negative control (larvae treated with acetone and tween 80 only in water).

Table 4.3

Aqueous leaf extract of *Carica papaya* yielded maximum mortality of 62%, 57% and 41.3% against *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* respectively at 48 hours. The ethanol extract of the leaves of *Carica papaya* showed 100% mortality against the larvae of *Culex quinquefasciatus* within 24 hours while maximal mortality

recorded was achieved at 48 hours against *Aedes vittatus* and *Anopheles gambiae* with 81.3% and 97.34% respecively. Hexane extract of the leaves of *Carica papaya* was effective against all three larvae with *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* having maximal mortality of 84%, 93.34% and 80% at 48 hours respectively. The ethanol extract of the leaves was insignificantly different ($p>0.05$) from positive control against *Anopheles gambiae* and *Culex quinquefasciatus*. This was mirrored by the effect of the hexane extract of the leaf against *Anopheles gambiae.*

Table 4.2: Percentage Mortality of *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* Treated with 500ppm (50mg/dl) of Aqueous, Ethanol and N-Hexane SEED Extracts of *Carica papaya.*

Extract Seeds	Time (hrs)	Percentage Mortality (%)		
		Aedes vittatus	*Anopheles gambiae*	*Culex quinquefasciatus*
Aqueous	12	12.00 ± 4.0^{e}	0 ± 0^{e}	1.30 ± 2.3^{e}
	24	13.30 ± 4.6^{e}	1.30 ± 2.3^{e}	5.30 ± 2.3^{e}
	48	13.30 ± 4.6^{e}	1.30 ± 2.3^{e}	5.30 ± 2.3^{e}
Ethanol	12	34.67 ± 9.2^{d}	28.00 ± 20.0^{d}	84 ± 0^{a}
	24	44.00 ± 4.0^{d}	45.34 ± 12.8^{d}	100.00 ± 0.0^{a}
	48	73.34 ± 2.3^{c}	76.00 ± 6.9^{bc}	100.00 ± 0.0^{a}
Hexane	12	70.60 ± 2.3^{c}	29.34 ± 18.0^{d}	33.34 ± 29.0^{d}
	24	77.30 ± 2.3^{b}	37.34 ± 12.8^{d}	46.67 ± 27.2^{d}
	48	98.67 ± 2.3^{ab}	92.00 ± 13.8^{ab}	72.00 ± 48.4^{c}
Control	12	100.00 ± 0^{a}	100.00 ± 0^{a}	100.00 ± 0^{a}
	24	100.00 ± 0^{a}	100.00 ± 0^{a}	100.00 ± 0^{a}
	48	100.00 ± 0^{a}	100.00 ± 0^{a}	100.00 ± 0^{a}
Control	12	0 ± 0	0 ± 0	0 ± 0
	24	0 ± 0	0 ± 0	0 ± 0
	48	0 ± 0	0 ± 0	0 ± 0

Test groups 1,2 and 3: Larvae of *Aedes vittatus, Anopheles gambiae* and *Culex quinquefasciatus* subjected to 500ppm (50mg/dl) of Aqueous, Ethanol and Hexane extracts of seed of *Carica papaya* respectively

Control: Larvae of the three species subjected to 3ml ethanol in water.

Control: Larvae of the three species subjected to 1ml of acetone and tween 80 in water only.

Values are Means±SD. Values with different subscripts are significantly ($p<0.05$) different

Table 4.3: Percentage Mortality of *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* Treated with 500ppm (50mg/dl) of Aqueous, Ethanol and N-Hexane LEAF Extracts of *Carica papaya.*

Extract Leaf	Time (hrs)	Percentage Mortality (%)		
		Aedes vittatus	*Anopheles gambiae*	*Culex quinquefasciatus*
Aqueous	12	26.67 ± 6.1^{hij}	10.60 ± 2.3^{lm}	18.60 ± 2.3^{jkl}
	24	40.00 ± 0.0^{fg}	38.00 ± 0.0^{fg}	36.00 ± 4.0^{fgh}
	48	62.00 ± 0.0^{c}	57.00 ± 0.0^{cd}	41.30 ± 4.6^{ef}
Ethanol	12	34.60 ± 6.1^{fgh}	25.34 ± 8.3^{hijk}	16.00 ± 0.0^{kl}
	24	48.00 ± 6.1^{de}	85.34 ± 10.5^{ab}	100.00 ± 0.0^{a}
	48	81.30 ± 2.3^{b}	97.34 ± 2.3^{a}	100.00 ± 0.0^{a}
Hexane	12	18.60 ± 6.1^{jkl}	29.34 ± 10.0^{ghi}	5.30 ± 6.1^{m}
	24	21.30 ± 4.6^{ijk}	40.00 ± 8.0^{efg}	8.00 ± 10.5^{m}
	48	84.00 ± 4.0^{b}	93.34 ± 8.7^{a}	80.00 ± 4.0^{b}
Control	12	100.00 ± 0^{a}	100.00 ± 0^{a}	100.00 ± 0^{a}
	24	100.00 ± 0^{a}	100.00 ± 0^{a}	100.00 ± 0^{a}
	48	100.00 ± 0^{a}	100.00 ± 0^{a}	100.00 ± 0^{a}
Control	12	0±0	0±0	0±0
	24	0±0	0±0	0±0
	48	0±0	0±0	0±0

Test groups 1, 2 and 3: Larvae of *Aedes vittatus, Anopheles gambiae* and *Culex quinquefasciatus* subjected to 500ppm (50mg/dl) of Aqueous, Ethanol and Hexane extracts of leaf of *Carica papaya* respectively
Control: Larvae of the three species subjected to 3ml ethanol in water.
Control: Larvae of the three species subjected to 1ml of acetone and tween 80 in water only.
Values are Means±SD. Values with different subscripts are significantly ($p<0.05$) different.

Table 4.4

The aqueous extract of the stem of *Carica papaya* gave mortality of 40%, 37.3% and 42.6% against *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* respectively while the ethanol extract of the stem gave mortality values of 40%, 36% and 40% against the three species. However none of the extracts of the stems of *Carica papaya* were able to reach the bench mark of 70% mortality against at least two of the three species.

Table 4.5

The aqueous, ethanol and hexane extract of the roots of *Carica papaya* were unable to induce any mortality when tested on the three mosquito species.

Table 4.4: Percentage Mortality of *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* Treated with 500ppm (50mg/dl) of Aqueous, Ethanol and N-Hexane STEM Extracts of *Carica papaya*.

Extract Stem	Time (hrs)	Percentage Mortality (%)		
		Aedes vittatus	*Anopheles gambiae*	*Culex quinquefasciatus*
Aqueous	12	21.30 ± 4.6^{c}	17.30 ± 2.3^{cd}	34.60 ± 4.6^{b}
	24	37.30 ± 2.3^{b}	26.60 ± 6.1^{c}	37.30 ± 4.6^{b}
	48	40.00 ± 8.0^{b}	37.30 ± 20.0^{b}	42.60 ± 12.0^{b}
Ethanol	12	24.00 ± 8.0^{c}	14.60 ± 2.3^{de}	24.00 ± 12.8^{c}
	24	34.6 ± 4.6^{b}	25.30 ± 2.3^{c}	36.00 ± 6.9^{b}
	48	40.00 ± 0.0^{b}	36.00 ± 4.0^{b}	40.00 ± 0.0^{b}
Hexane	12	4.00 ± 4.0^{e}	9.30 ± 8.3^{de}	0 ± 0^{e}
	24	6.60 ± 2.3^{de}	14.60 ± 10.0^{de}	4.00 ± 4.0^{e}
	48	10.60 ± 4.6^{de}	20.00 ± 4.0^{c}	8.00 ± 4.6^{de}
Control	12	100.00 ± 0^{a}	100.00 ± 0^{a}	100.00 ± 0^{a}
	24	100.00 ± 0^{a}	100.00 ± 0^{a}	100.00 ± 0^{a}
	48	100.00 ± 0^{a}	100.00 ± 0^{a}	100.00 ± 0^{a}
Control	12	0±0	0±0	0±0
	24	0±0	0±0	0±0
	48	0±0	0±0	0±0

Test groups 1, 2 and 3: Larvae of *Aedes vittatus, Anopheles gambiae* and *Culex quinquefasciatus* subjected to 500ppm (50mg/dl) of Aqueous, Ethanol and Hexane extracts of stem of *Carica papaya* respectively

Control: Larvae of the three species subjected to 3ml ethanol in water.

Control: Larvae of the three species subjected to 1ml of acetone and tween 80 in water only. Values are Means±SD.

Values with different subscripts are significantly ($p<0.05$) different.

Table 4.5: Percentage Mortality of *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* Treated with 500ppm (50mg/dl) of Aqueous, Ethanol and N-Hexane ROOT Extracts of *Carica papaya.*

Extract Roots	Time (hrs)	Percentage Mortality (%) *Aedes vittatus*	*Anopheles gambiae*	*Culex quinquefasciatus*
Aqueous	12	0±0	0±0	0±0
	24	0±0	0±0	0±0
	48	0±0	0±0	0±0
Ethanol	12	0±0	0±0	0±0
	24	0±0	0±0	0±0
	48	0±0	0±0	0±0
Hexane	12	0±0	0±0	0±0
	24	0±0	0±0	0±0
	48	0±0	0±0	0±0
Control	12	100.00±0[a]	100.00±0[a]	100.00±0[a]
	24	100.00±0[a]	100.00±0[a]	100.00±0[a]
	48	100.00±0[a]	100.00±0[a]	100.00±0[a]
Control	12	0±0	0±0	0±0
	24	0±0	0±0	0±0
	48	0±0	0±0	0±0

Test groups 1, 2 and 3: Larvae of *Aedes vittatus, Anopheles gambiae* and *Culex quinquefasciatus* subjected to 500ppm (50mg/dl) of Aqueous, Ethanol and Hexane extracts of root of *Carica papaya* respectively
Control: Larvae of the three species subjected to 3ml ethanol in water.
Control: Larvae of the three species subjected to 1ml of acetone and tween 80 in water only. Values are Means±SD.
Values with different subscripts are significantly ($p<0.05$) different.

Table 4.6

Aqueous seed extract of *Dacryodes edulis* did not show significant (($p>0.05$) larval mortality against the larvae of *Aedes vittatus, Anopheles gambiae* and *Culex quinquefasciatus*. Maximal death at 48 hours was 10.67% against *Anopheles gambiae* and *Culex quinquefasciatus* while maximal mortality achieved against *Aedes vittatus* was 8%.

The ethanol extract of the seeds of *Dacryodes edulis* showed 68% mortality against the larvae of *Culex quinquefasciatus* within the first 12 hours and maximal mortality of 74.6% at 48hrs. The extract caused mortality of 93.3% at 48hrs against *Anopheles gambiae* and maximal mortality of 70.6% against *Aedes vittatus* at 48 hours.

Hexane extract of the seeds of *Dacryodes edulis* did not show significant ($p>0.05$) mortality against the larvae of *Anopheles gambiae* with maximal mortality of 21% being achieved at 48 hours and mortality values of 49.3% against the larvae of *Aedes vittatus*. However, there was significant ($p<0.05$) mortality observed in the *Culex quinquefasciatus* group with larval mortality of 74.67% at 48 hours when compared to the effects on *Aedes vittatus* and *Anopheles gambiae*.

4.6: Percentage Mortality of *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* Treated with 500ppm (50mg/dl) of Aqueous, Ethanol and N-Hexane SEED Extracts of *Dacryodes edulis*.

Extract Seed	Time (hrs)	Percentage Mortality (%)		
		Aedes vittatus	*Anopheles gambiae*	*Culex quinquefasciatus*
Aqueous	12	4.00±4.00^{i}	8.00±8.00^{hi}	8.00±4.00^{hi}
	24	4.00±4.00^{i}	9.30±6.10^{hi}	8.00±4.00^{hi}
	48	8.00±4.00^{hi}	10.67±8.30^{hi}	10.60±8.30^{hi}
Ethanol	12	38.67±10.00^{def}	49.30±10.00^{cd}	68.00±21.10^{b}
	24	44.00±6.90^{de}	80.00±20.00^{b}	69.30±22.00^{b}
	48	70.60±12.20^{b}	93.30±8.30^{a}	74.60±12.85^{b}
Hexane	12	29.34±8.30^{efg}	12.00±4.00^{hi}	25.30±6.10^{fgh}
	24	34.67±4.60^{def}	20.00±10.58^{ghi}	62.67±16.10^{bc}
	48	49.30±2.30^{cd}	21.30±10.00^{gh}	74.67±12.80^{b}
Control	12	100.00±0^{a}	100.00±0^{a}	100.00±0^{a}
	24	100.00±0^{a}	100.00±0^{a}	100.00±0^{a}
	48	100.00±0^{a}	100.00±0^{a}	100.00±0^{a}
Control	12	0±0	0±0	0±0
	24	0±0	0±0	0±0
	48	0±0	0±0	0±0

Test groups 1, 2 and 3: Larvae of *Aedes vittatus, Anopheles gambiae* and *Culex quinquefasciatus* subjected to 500ppm (50mg/dl) of Aqueous, Ethanol and Hexane extracts of seed of *Dacryodes edulis* respectively

Control: Larvae of the three species subjected to 3ml ethanol in water.

Control: Larvae of the three species subjected to 1ml of acetone and tween 80 in water only. Values are Means±SD.

Values with different subscripts are significantly ($p<0.05$) different.

Table 4.7

Aqueous leaves extract of *Dacryodes edulis* did not show significant (p>0.05) larval mortality against *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus*. Maximal death at 48 hours was 10.6% against *Aedes vittatus* and *Anopheles gambiae* and 12% against *Culex quinquefasciatus*.

The ethanol extract of the leaves of *Dacryodes edulis* showed 29.34% mortality against the larvae of *Aedes vittatus* and *Anopheles gambiae* within the first 12 hours. *Aedes vittatus* gave mortality values of 32% and 40% at 24 hours and 48 hours respectively. *Anopheles gambiae* exhibited mortality of 37.34% at 24 hours with no increase in mortality up to 48 hours. Highest mortality recorded against *Culex quinquefasciatus* was achieved at 48 hours with 82.67%.

Hexane extract of the leaf of *Dacryodes edulis* induced larval mortality of 25.3%, 34.67% and 70.67% at 12 hours, 24 hours and 48 hours against *Aedes vittatus;* 28.4% at 12 hours, 53.3% at 24hours and 90.6% at 48 hours against *Anopheles gambiae.* The hexane extract tested against the larvae of *Culex quinquefasciatus* showed mortality of 26.67% at 12 hours, 50.67% at 24 hours and 93.3% at 48 hours. The percent mortality at 48 hours against *Anopheles gambiae* and *Culex quinquefasciatus* was comparable to the mortality achieved with the positive control.

Table 4.7: Percentage Mortality of *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* Treated with 500ppm (50mg/dl) of Aqueous, Ethanol and N-Hexane LEAF Extracts of *Dacryodes edulis*.

Extract Leaf	Time (hrs)	Percentage Mortality (%)		
		Aedes vittatus	*Anopheles gambiae*	*Culex quinquefasciatus*
Aqueous	12	2.60 ± 2.30^{f}	8.00 ± 4.00^{f}	8.00 ± 4.00^{f}
	24	8.00 ± 4.00^{f}	8.00 ± 4.00^{f}	9.30 ± 4.00^{f}
	48	10.60 ± 4.00^{f}	10.67 ± 4.60^{f}	12.00 ± 4.00^{f}
Ethanol	12	29.34 ± 4.60^{e}	29.34 ± 4.60^{e}	38.67 ± 22.00^{e}
	24	32.00 ± 0.00^{e}	37.34 ± 4.60^{e}	49.3 ± 23.40^{d}
	48	40.00 ± 8.00^{d}	37.34 ± 4.60^{e}	82.67 ± 12.20^{bc}
Hexane	12	25.33 ± 2.30^{e}	28.00 ± 4.00^{e}	26.67 ± 6.10^{e}
	24	34.67 ± 12.20^{e}	53.30 ± 11.54^{d}	50.67 ± 10.00^{d}
	48	70.67 ± 2.30^{c}	90.60 ± 6.10^{ab}	93.30 ± 11.50^{a}
Control	12	100.00 ± 0^{a}	100.00 ± 0^{a}	100.00 ± 0
	24	100.00 ± 0^{a}	100.00 ± 0^{a}	100.00 ± 0^{a}
	48	100.00 ± 0^{a}	100.00 ± 0^{a}	100.00 ± 0^{a}
Control	12	0±0	0±0	0±0
	24	0±0	0±0	0±0
	48	0±0	0±0	0±0

Test groups 1, 2 and 3: Larvae of *Aedes vittatus, Anopheles gambiae* and *Culex quinquefasciatus* subjected to 500ppm (50mg/dl) of Aqueous, Ethanol and Hexane extracts of leaf of *Dacryodes edulis* respectively
Control: Larvae of the three species subjected to 3ml ethanol in water.
Control: Larvae of the three species subjected to 1ml of acetone and tween 80 in water only. Values are Means±SD.
Values with different subscripts are significantly ($p<0.05$) different.

Table 4.8

Aqueous ethanol and hexane stem extracts were unable to cause any mortality in the larvae of *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus.*

Table 4.9

Aqueous ethanol and hexane root extracts were unable to cause any mortality in the larvae of *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus.*

Table 4.8: Percentage Mortality of *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* Treated with 500ppm (50mg/dl) of Aqueous, Ethanol and N-Hexane STEM Extracts of *Dacryodes edulis*.

Extract Stem	Time (hrs)	Percentage Mortality (%)		
		Aedes vittatus	*Anopheles gambiae*	*Culex quinquefasciatus*
Aqueous	12	0±0	0±0	0±0
	24	0±0	0±0	0±0
	48	0±0	0±0	0±0
Ethanol	12	0±0	0±0	0±0
	24	0±0	0±0	0±0
	48	0±0	0±0	0±0
Hexane	12	0±0	0±0	0±0
	24	0±0	0±0	0±0
	48	0±0	0±0	0±0
Control	12	100.00±0	100.00±0	100.00±0
	24	100.00±0	100.00±0	100.00±0
	48	100.00±0	100.00±0	100.00±0
Control	12	0±0	0±0	0±0
	24	0±0	0±0	0±0
	48	0±0	0±0	0±0

Test groups 1, 2 and 3: Larvae of *Aedes vittatus, Anopheles gambiae* and *Culex quiquefasciatus* subjected to 500ppm (50mg/dl) of Aqueous, Ethanol and Hexane extracts of stems of *Dacryodes edulis* respectively

Control: Larvae of the three species subjected to 3ml ethanol in water.

Control: Larvae of the three species subjected to 1ml of acetone and tween 80 in water only. Values are Mean±SD.

Values with different subscripts are significantly ($p<0.05$) different.

Table 4.9: Percentage Mortality of *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* Treated with 500ppm (50mg/dl) of Aqueous, Ethanol and N-Hexane ROOT Extracts of *Dacryodes edulis*.

Extract Root	Time (hrs)	Percentage Mortality (%) *Aedes vittatus*	*Anopheles gambiae*	*Culex quinquefasciatus*
Aqueous	12	0±0	0±0	0±0
	24	0±0	0±0	0±0
	48	0±0	0±0	0±0
Ethanol	12	0±0	0±0	0±0
	24	0±0	0±0	0±0
	48	0±0	0±0	0±0
Hexane	12	0±0	0±0	0±0
	24	0±0	0±0	0±0
	48	0±0	0±0	0±0
Control	12	100.00±0	100.00±0	100.00±0
	24	100.00±0	100.00±0	100.00±0
	48	100.00±0	100.00±0	100.00±0
Control	12	0±0	0±0	0±0
	24	0±0	0±0	0±0
	48	0±0	0±0	0±0

Test groups 1, 2 and 3: Larvae of *Aedes vittatus, Anopheles gambiae* and *Culex quiquefasciatus* subjected to 500ppm (50mg/dl) of Aqueous, Ethanol and Hexane extracts of roots of *Dacryodes edulis* respectively
Control: Larvae of the three species subjected to 3ml ethanol in water.
Control: Larvae of the three species subjected to 1ml of acetone and tween 80 in water only. Values are Means±SD.
Values with different subscripts are significantly ($p<0.05$) different.

4.2.2 Assay II

LC_{50} and LC_{90}Determination

4.2.2.1 Carica papaya

The percentage changes in the mortality of different concentrations of ethanolic and hexane extracts of the leaves and seedsof *Carica papaya* against *Aedes vittatus, Anopheles gambiae* and *Culex quinquefasciatus* were recorded and used to determine LC_{50} and LC_{90} values at 12, 24 and 48 hours. These are shown in Table 4.10, 4.11 and 4.12.

Table 4.10

When *Carica papaya* extracts were tested against *Aedes vittatus* the ethanolic extract of the leaves gave LC_{50} values of 208.4, 167.1 and 93.1ppm with LC_{90}values of 2405.0, 2028.0 and 1625.0ppm at 12, 24 and 48 hours respectively. The hexane extract of the leaves gave LC_{50} values of 837.2, 120.6 and 48.1ppm at 12, 24 and 48hours. The corresponding LC_{90} values for the same time period were 51608.0, 1314.0 and 456.4ppm. The ethanolic extract of the seeds of *Carica papaya* yielded LC_{50} values of 133.2, 112.1 and 100.0ppm and LC_{90} values of 690.8, 658.4 and 654.6ppm at 12, 24 and 48 ours respectively while the hexane extract of the seeds gave LC_{50} values of 231.4, 119.7 and 61.4ppm with LC_{90} values 3885.4, 1078.4 and 466.8ppm at 12, 24 and 48 hours.

Table 4.11

The ethanol and hexane extracts of the leaves of *Carica papaya* gave LC_{50} values at 12, 24 and 48 hours of 133.7, 75.5, 40.8ppm and 1567.3, 53.3 and 29.3ppm. The LC_{90} values for the corresponding time period were 690.8, 250.0, 313.3ppm and 64453.0, 2090.0, 672.2ppm when tested against *Anopheles gambiae*. The ethanol extact of the seeds of the plant gave LC_{50} values of 150.8, 71.0 and 62.2ppm at 12, 24 and 48 hours while the LC_{90}

values were 887.0, 239.9 and 202.0ppm over the same time period. The hexane extract of the seeds produced LC_{50} and LC_{90} values of 851.5 and 8626.0ppm at 12 hours, 142.9 and 677.0ppm at 24 hours and 111.7 and 536.9ppm at 48 hours.

Table 4.12

The ethanol extract of the seeds of *Carica papaya* against *Culex quinquefasciatus* gave LC_{50} values of 1151.2, 815.1 and 262.1ppm with corresponding LC_{90} values of 8705.0, 7050.0 and 882.8ppm at 12, 24 and 48 hours. The hexane extract of the seeds which gave LC_{50} values of 417.8, 307.2 and 264.7ppm at 12, 24 and 48 hours similar to the result derived from the ethanol extract of the seeds at maximum time of 48 hours.

Against *Culex quinquefascatus* at 12, 24 and 48 hours the ethanol extract of the leaves was shown to have LC_{50} values of 1541.8, 130.3 and 43.9ppm and LC_{90} values of 1271.9ppm at 48 hours. The hexane extract of the leaves gave LC_{50} values of 268.8, 129.6 and 74.8ppm at 12, 24 and 48 hours while LC_{90} value at 48 hours was 801.2ppm.

Table 4.10: Percentage Mortality and Lethal Concentrations of *Carica papaya* (Ethanol and Hexane) Seed and (Ethanol and Hexane) Leaf Extracts against *Aedes vittatus*.

Extract	Conc (ppm)	6.25	12.5	25	50	100	200	400	Lethal Concentration (ppm)		X^2	R^2
	Time (hrs)	**Percentage Mortality(%)**							**LC_{50} (UCL-LCL)**	**LC_{90} (UCL-LCL)**		
CPESE	12	4.0±0.0	-	8.0±0.0	16.0±0.0	52.0±4.0	60.0±4.0	80.0±8.0	**133.2** (552.3-137.4)	**690.8** (1593.7-414.6)	5.668	0.933
	24	4.0±0.0	8.0±4.0	8.0±0.0	16.0±0.0	60.0±4.6	68.0±0.0	80.0±8.0	**112.1** (161.8-82.0)	**658.4** (1547.4-338.1)	5.303	0.924
	48	8.0±4.0	8.0±4.0	8.0±4.0	20.0±4.0	64.0±2.3	74.0±4.0	80.0±0.0	**100.0** (145.5-72.3)	**654.6** (1591.2-377.7)	7.624	0.886
CPHSE	12	8.0±4.0	12.0±4.0	12.0±0.0	12.0±0.0	40.0±6.1	52.0±4.6	60.0±0.0	**231.4** (552.3-137.4)	**3885.4** (40534.0-1252.2)	3.466	0.875
	24	8.0±0.0	12.0±4	16.0±4.0	16.0±0.0	48.0±4.0	64.0±6.4	80.0±8.0	**119.7** (192.4-82.6)	**1078.4** (3660.0-533.83)	3.953	0.917
	48	12.0±4.0	24.0±4.0	24.0±0.0	24.0±4.0	60.0±0.0	80.0±4.0	96.0±4.0	**61.4** (122.3-33.7)	**466.8** (3442.0-201.7)	8.311	0.880
CPELE	12	8.0±0.0	8.0±4.0	12.0±4.0	16.0±4.0	32.0±4.0	33.4±23.0	80.0±17.4	**208.4** (420.0-132.3)	**2405.3** (14430.0-946.8)	7.249	0.844
	24	8.0±0.0	12.0±0.0	12.0±4	24.0±4.0	36.0±4.0	44.0±23.4	80.0±17.4	**167.1** (316.9-108.0)	**2028.1** (11206.0-823.1)	8.815	0.901
	48	16.0±4.0	20.0±8.0	24.0±0.0	36.0±14.4	44.0±0.0	60.0±18.9	84.0±14.4	**93.1** (166.1-59.5)	**1625.0** (10095.0-635.8)	2.749	0.923
CPHLE	12	4.0±4.0	8.0±4.0	20.0±0.0	20.0±4.0	24.0±0.0	32.0±0.0	40.0±4.0	**837.2** (16807.0-298.7)	**51608.7** (108712688.0-4875.6)	1.192	0.927
	24	8.0±4.0	12.0±4.0	20.0±4.0	28.0±8.0	20.0±4.0	20.0±4.0	80.0±4.0	**120.6** (203.2-81.1)	**1314.3** (5430.0-599.6)	1.290	0.972
	48	8.0±4.0	40.0±6.9	36.0±10.5	40.0±4.0	48.0±6.9	80.0±4.0	100.0±0.0	**48.144** (129.9-18.2)	**456.44** (2307.0-156.5)	13.14	0.711

CPESE- *Carica papaya* ethanol seed extract, CPHSE- *Carica papaya* hexane seed extract, CPELE- *Carica papaya* ethanol leaf extract, CPHLE- *Carica papaya* hexane leaf extract. Mortality values represent Means±SD of 3 replicatates of r=25

Table 4.11: Percentage Mortality and Lethal Concentrations of *Carica papaya* (Ethanol and Hexane) Seed and (Ethanol and Hexane) Leaf Extracts against *Anopheles gambiae*.

Extract	Conc (ppm)	6.25	12.5	25	50	100	200	400	Lethal Concentration (ppm)		X^2	R^2
	Time (hrs)	**Percentage Mortality (%)**							**LC_{50} (UCL-LCL)**	**LC_{90} (UCL-LCL)**		
CPESE	12	4.0±0.0	4.0±4.0	4.0±4.0	12.0±4.0	48.0±4.0	64.0±4.0	70.0±8.0	**150.8** (226.9-109.0)	**887.0** (2327.7-499.6)	5.819	0.894
	24	4.0±0.0	8.0±0.0	12.0±4.0	20.0±4.0	48.0±4.0	100.0±0.0	100.0±0.0	**71.0** (162.8-36.0)	**239.9** (2144.0-117.9)	17.869	0.938
	48	4.0±0.0	8.0±0.0	12.0±4.0	28.0±6.9	60.0±6.9	100.0±4.0	100.0±0.0	**62.24** (111.9-36.8)	**202.05** (813.2-112.2)	12.123	0.941
CPHSE	12	-	4.0±4.0	4.0±0.0	4.0±0.0	8.0±4.0	8.0±4.0	49.3±6.9	**851.5** (7597.0-408.7)	**8626.0** (359757.0-2133.0)	8.012	0.641
	24	-	8.0±4.0	12.0±4.0	16.0±4.0	20.0±0.0	48.0±4.6	100.0±0.0	**142.9** (815.5-69.7)	**677.0** (36010.0-257.4)	16.340	0.870
	48	-	12.0±4.0	12.0±0.0	24.0±6.9	24.0±0.0	64.0±4.0	100.0±0.0	**111.7** (321.2-56.6)	**536.9** (10750.0-220.5)	14.783	0.796
CPELE	12	-	12.0±4.0	16.0±4.0	20.0±4.0	28.0±4.0	48.0±0.0	95.0±6.9	**133.7** (447.7-67.9)	**690.8** (26105.0-297.0)	12.534	0.762
	24	12.0±4.0	12.0±0.0	28.0±4.0	36.0±6.9	48.0±4.0	60.0±8.0	100.0±4.0	**75.5** (169.5-40.2)	**250.0** (7414.4-247.0)	8.794	0.966
	48	20.0±0.0	24.0±8.0	36.0±0.0	36.0±6.9	71.3±10.9	84.0±8.0	100.0±0.0	**40.8** (57.6-28.6)	**313.32** (727.0-185.9)	7.348	0.896
CPHLE	12	-	4.0±4.0	12.0±0.0	16.0±4.0	16.0±4.0	20.0±8.0	32.0±6.9	**1567.3** (75812.0-477.7)	**64453.0** (350907952.0-5580.0)	2.090	0.878
	24	20.0±4.0	36.0±8.0	48.0±4.0	40.0±4.0	48.0±0.0	71.3±10.9	80.0±4.0	**53.3** (99.4-29.3)	**2090.1** (32586.9-634.9)	3.627	0.879
	48	36.0±4.0	40.0±4.0	48.0±0.0	52.0±4.0	56.0±8.0	76.0±4.0	100.0±0.0	**29.3** (727.0-185.9)	**672.2** (107520.0-189.5)	8.633	0.856

CPESE- *Carica papaya* ethanol seed extract, CPHSE- *Carica papaya* hexane seed extract, CPELE- *Carica papaya* ethanol leaf extract, CPHLE- *Carica papaya* hexane leaf extract. Mortality values represent Means±SD of 3 replicatates of r=25

Table 4.12: Percentage Mortality and Lethal Concentrations of *Carica papaya* (Ethanol and Hexane) Seed and (Ethanol and Hexane) Leaf Extracts against *Culex quinquefasciatus*.

Extract	Conc (ppm)	6.25	12.5	25	50	100	200	400	Lethal Concentration (ppm)		X^2	R^2
	Time (hrs)	Percentage Mortality (%)							LC_{50} (UCL-LCL)	LC_{90} (UCL-LCL)		
CPESE	12	-	-	-	-	12.0±4.0	16.0±4.0	20.0±4.0	**1151.2** (24180.0-504.6)	**8705.0** (4069326.0-1879.0)	2.890	0.998
	24	-	-	4.0±0.0	4±4	12.0±4.0	16.0±0.0	36.0±8.0	**815.1** (4850.0-405.0)	**7050.0** (307863.0-1852.0)	1.210	0.924
	48	-	-	4.0±0.0	4±4	12.0±0.0	24.0±4.0	80.0±0.0	**262.1** (682.3-163.8)	**882.8** (10726.0-426.1)	8.679	0.845
CPHSE	12	-	-	-	-	-	24.0±0.0	43.3±6.9	**417.8** (786.6-312.9)	**1130.0** (5428.0-652.6)	1.901	0.99
	24	-	-	-	-	12.0±0.0	32.0±4.0	60.0±4.0	**307.2** (464.7-236.0)	**903.3** (2518.1-561.8)	0.765	0.99
	48	-	-	-	4.0±4.0	12.0±0.0	32.0±4.0	60.0±4.0	**264.7** (218.4-34.5)	**774.9** (34837.0-256.1)	0.742	0.981
CPELE	12	-	-	-	-	16.0±4.0	16.0±0.0	16.0±4.6	**1541.8** (82292.0-567.7)	**16151.0** (42904892.0-2547.0)	5.477	0.99
	24	-	-	16.0±4.0	32.0±6.9	32.0±0.0	64.0±6.9	80.0±0.0	**130.3** (188.3-96.1)	**699.5** (1678.0-413.7)	4.177	0.938
	48	16.0±4.0	16.0±0.0	32.0±4.0	40.0±0.0	80.0±14.4	80.0±2.3	100.0±0.0	**43.9** (60.4-31.8)	**271.9** (557.3-171.1)	6.950	0.906
CPHLE	12	-	4.0±0.0	8.0±4.0	12.0±0.0	12.0±4.0	20.0±4.0	84.0±10.9	**268.8** (4372.0-124.0)	**1539.9** (2819182.0-441.6)	14.609	0.719
	24	-	12.0±0.0	28.0±0.0	28.0±4.0	36.0±0.0	44.0±4.0	88.0±6.9	**129.6** (415.9-66.8)	**1188.9** (40575.0-384.0)	9.235	0.805
	48	12.0±0.0	20.0±4.0	32.0±4.0	36.0±4.0	40.0±4.0	60.0±0.0	100.0±4.0	**74.8** (218.4-34.5)	**801.2** (34837.0-256.1)	10.518	0.954

CPESE- *Carica papaya* ethanol seed extract, CPHSE- *Carica papaya* hexane seed extract, CPELE- *Carica papaya* ethanol leaf extract, CPHLE- *Carica papaya* hexane leaf extract. Mortality values represent Means±SD of 3 replicatates of r=25

4.2.2.2 *Dacryodes edulis*

The percentage changes in the mortality of different concentrations of ethanolic and hexane extracts of the seed and leaf of *Dacryodes edulis* against *Aedes vittatus, Anopheles gambiae* and *Culex quinquefasciatus* were recorded and used to determine LC_{50} and LC_{90} values at 12, 24 and 48 hours. These are shown in Table 4.13, 4.14 and 4.15.

Table 4.13

The ethanolic extract of the seeds of *Dacryodes edulis* yielded LC_{50} values of 317.9, 172.1 and 150.5ppm and LC_{90} value of 1876.0ppm at 48 hours against *Aedes vittatus* and the hexane extract of the leaves gave LC_{50} values of 2618.3, 1943.8 and 1177.0ppm at 12, 24 and 48 hours.

Table 4.14

The ethanol extract of the seed and hexane extract of the leaf of *Dacryodes edulis* gave LC_{50} values at 12, 24 and 48 hours of 235.4, 137.0 and 85.9ppm and 2164.0, 1929.6 and 1762.0ppm respectively when tested against *Anopheles gambiae.* The LC_{90} value at 48 hours of the ethanol extract of the seeds of the plant gave a value of 407.9ppm.

Table 4.15

Against *Culex quinquefascatus* the ethanol extract of the seeds gave LC_{50} values of 231.1, 167.0 and 110.1ppm at 12, 24 and 48 hours respectively with LC_{90} value of 591.5ppm at 48 hours and the hexane extract of the leaf was shown to have an LC_{50} value of 971.9ppm at both 12 hours and 24 hours with a lower value of 508.2ppm at 48 hours.

Table 4.13: Percentage Mortality and Lethal Concentrations of *Dacryodes edulis* Ethanol Seed Extract and Hexane Leaf Extract against *Aedes vittatus*.

Aedes vittatus

Extract	Conc (ppm)	6.25	12.5	25	50	100	200	400	Lethal Concentration (ppm)		X^2	R^2
	Time (hrs)	Percentage Mortality (%)							LC_{50} (UCL-LCL)	LC_{90} (UCL-LCL)		
DEESE	12	-	4.0±0.0	8.0±0.0	12.0±0.0	20.0±0.0	36.0±6.9	60.0±4.0	**317.9** (684.9-201.9)	**2583.6** (15971.0-1049.0)	1.178	0.971
	24	-	16.0±4.0	20.0±6.9	24.0±4.0	32.0±0.0	40.0±4.0	80.0±4.0	**172.1** (325.9-111.8)	**1984.9** (10938.0-812.8)	7.427	0.809
	48	-	20.0±0.0	24.0±4.0	24.0±4.0	32.0±0.0	48.0±10.9	80±0.0	**150.5** (229.6-97.9)	**1876.0** (10386.4-765.7)	7.804	0.800
DEHLE	12	-	4.0±4.0	4.0±0.0	4.0±0.0	16.0±0.0	20.0±6.9	20.0±10.9	**2618.3** (541868.0-667.3)	**83869.0** (4272172201.0-6100.0)	1.916	0.822
	24	-	4.0±0.0	8.0±4.0	8.0±0.0	16.0±0.0	24.0±4.0	24.0±0.0	**1943.8** (145288.0-557.8)	**68192.0** (648743017.0-5708.0)	1.261	0.940
	48	-	4.0±0.0	8.0±4.0	16.0±0.0	16.0±0.0	24.0±4.0	32.0±0..0	**1177.0** (21712.0-426.1)	**32740.0** (21047816.0-4109.0)	1.259	0.958

DEESE- *Dacryodes edulis* ethanol seed extract and DEHLE- *Dacryodes edulis* hexane leaf extract
Mortality values represent Means±SD of 3 replicatates of r=25

Table 4.14: Percentage Mortality and Lethal Concentrations of *Dacryodes edulis* Ethanol Seed Extract and Hexane Leaf Extract against *Anopheles gambiae*.

Anopheles gambiae

Extract	Conc (ppm)	6.25	12.5	25	50	100	200	400	Lethal Concentration (ppm)		X^2	R^2
	Time (hrs)	Percentage Mortality (%)							LC_{50} (UCL-LCL)	LC_{90} (UCL-LCL)		
DEESE	12	-	8.0±4.0	8.0±0.0	20.0±0.0	20.0±0.0	40.0±10.0	70.6±10.0	**235.4** (442.4-156.0)	**1898.0** (8828.0-843.8)	4.365	0.890
	24	-	20.0±8.0	20.0±4.0	20.0±0.0	29.3±4.6	53.3±6.1	84.0±6.9	**137.0** (216.8-96.2)	**1024.6** (3228.0-529.0)	5.400	0.890
	48	4.0±4.0	20.0±8.0	20.0±4.0	26.6±4.6	37.0±8.3	53.3±6.1	100.0±0.0	**85.9** (154.9-52.8)	**407.9** (1805.0-208.0)	8.265	0.935
DEHLE	12	4.0±0.0	4.0±4.0	4.0±0.0	8.0±0.0	8.0±0.0	8.0±0.0	16.0±0.0	**2164.0** (197169.0-611.2)	**62347.0** (667456722.0-5428.0)	0.695	0.965
	24	4.0±0.0	4.0±0.0	8.0±4.0	8.0±0.0	8.0±0.0	16.0±4.0	16.0±0.0	**1929.6** (123114.0-566.0)	**60335.0** (370902372.0-5428.0)	0.930	0.959
	48	4.0±0.0	4.0±0.0	8.0±4.0	8.0±0.0	16.0±4.0	16.0±4.0	16.0±0.0	**1762.1** (88361.0-537.0)	**54815** (223420899.0-5200.0)	0.890	0.968

DEESE- *Dacryodes edulis*ethanol seed extract and DEHLE-*Dacryodes edulis* hexane leaf extract
Mortality values represent Means±SD of 3 replicates of r=25

Table 4.15: Percentage Mortality and Lethal Concentrations of *Dacryodes edulis* Ethanol Seed Extract and Hexane Leaf Extract against *Culex quinquefasciatus*.

Culex quinquefasciatus

Extract	Conc (ppm)	6.25	12.5	25	50	100	200	400	Lethal Concentration (ppm)		X^2	R^2
	Time (hrs)	Percentage Mortality (%)							LC_{50} (UCL-LCL)	LC_{90} (UCL-LCL)		
DEESE	12	-	8.0±0.0	8.0±0.0	16.0±0.0	25.3±9.2	32±6.9	76.3±16.0	**231.1** (425.6-154.5)	**1780.9** (7834.0-810.4)	5.793	0.868
	24	-	8.0±0.0	16.0±4.0	20.0±4.0	28.0±0.0	32.0±4.0	88.0±12.0	**167.0** (285.6-113.7)	**1395.0** (5333.0-662.1)	7.834	0.869
	48	-	8.0±0.0	16.0±4.0	20.0±4.0	30.6±9.2	40.0±0.0	98.67±9.2	**110.1** (253.1-61.8)	**591.5** (5448.0-256.2)	10.377	0.963
DEHLE	12	-	-	-	-	-	8.0±0.0	16.0±4.0	**971.9** (2508692.0-499.0)	**3396.0** (19336156455.0-1020.0)	0.735	0.99
	24	-	-	-	-	-	8.0±0.0	16.0±4.0	**971.9** (2508692.0-499.0)	**3396.0** (19336156455.0-1020.0)	0.735	0.99
	48	-	-	-	-	-	16.0±4.0	16.0±4.0	**508.2** (1376.5-360.6)	**1417.7** (14762.0-734.4)	0.979	0.99

DEESE- *Dacryodes edulis* ethanol seed extract and DEHLE- *Dacryodes edulis* hexane leaf extract
Mortality values represent Means±SD of 3 replicates of r=25

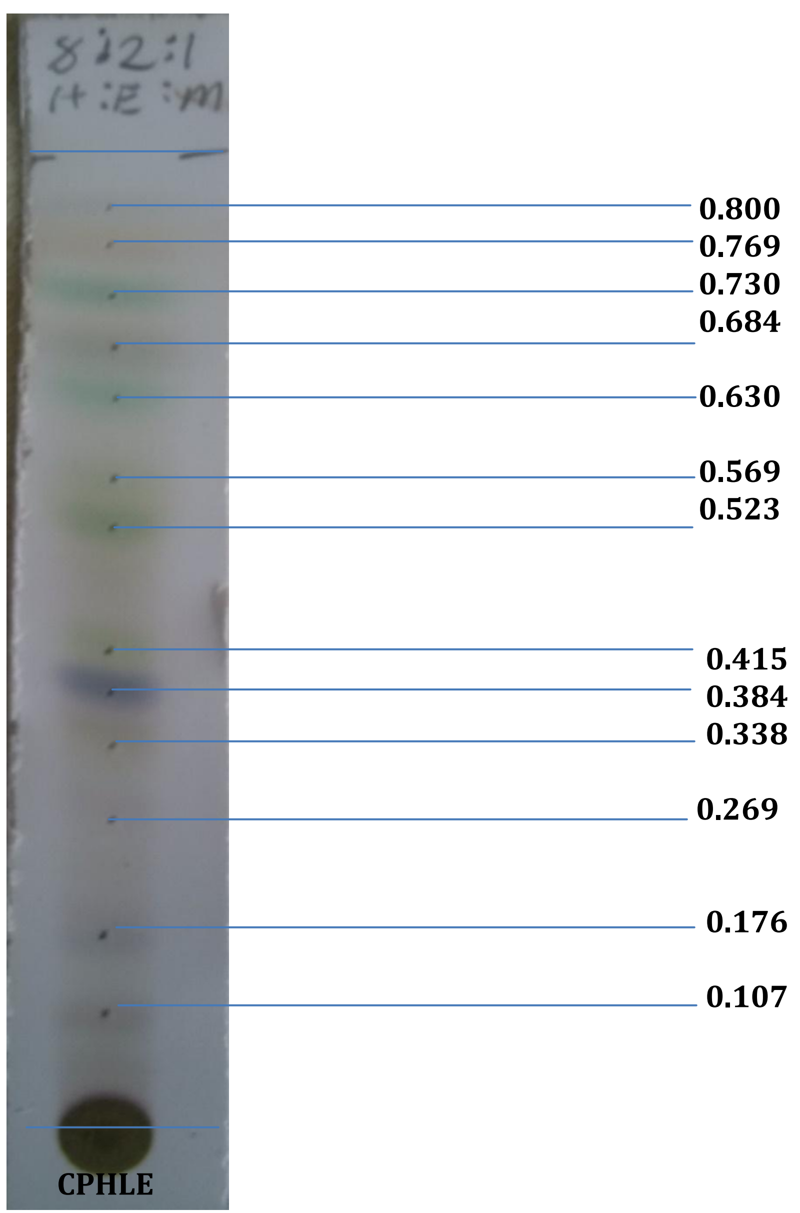

Plate II: TLC Separation of *Carica papaya* hexane leaf extract (CPHLE) using Hexane:Ethylacetate:Methanol (8:2:1).
Retention factor (R_f) of different bands using 10% sulphuric acid to visualize.

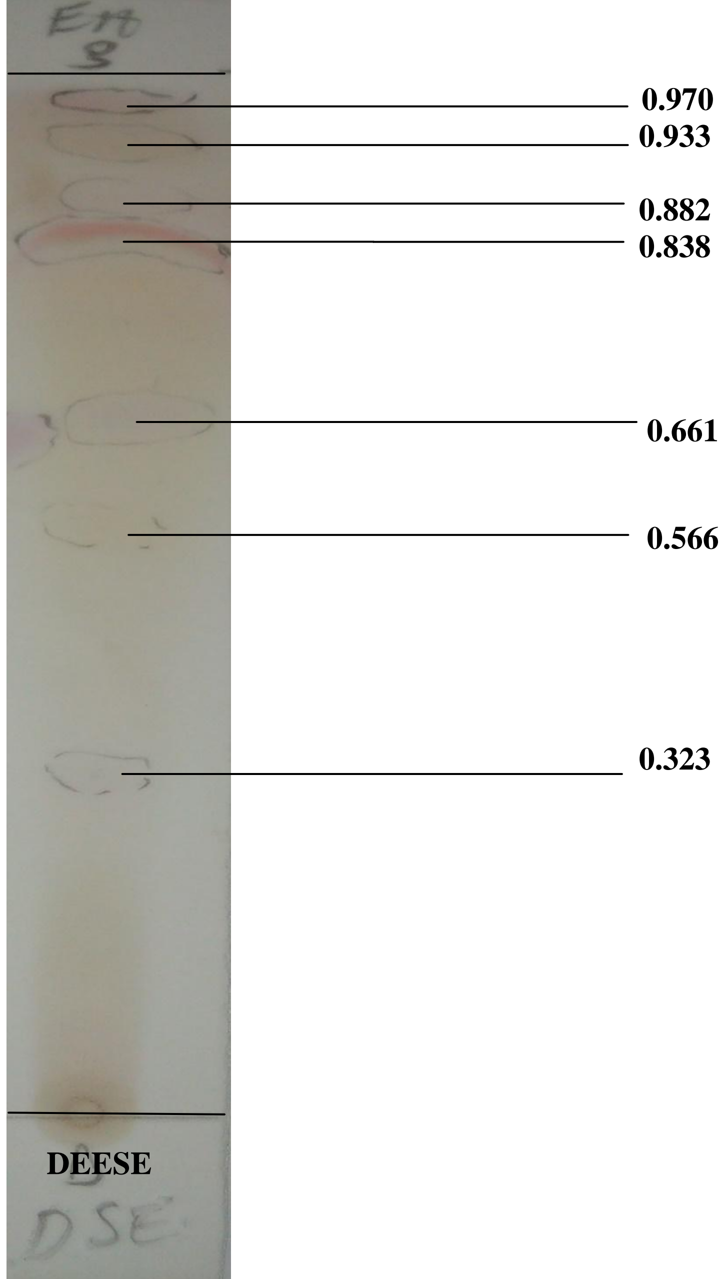

Plate III: TLC Separation of *Dacryodes edulis* ethanol seed extract (DEESE) using Chloroform: Ethylacetate (7:3).

Retention factor (R_f) of different bands using 10% sulphuric acid to visualize.

4.3 Larvicidal Testing

4.3.1 Assay III

The percentage changes in the mortality of different concentrations of fractions of the hexane extract of the leaves of *Carica papaya* and the ethanol extract of seed of *Dacryodes edulis* against *Aedes vittatus* and *Culex quinquefasciatus* were recorded and used to determine LC_{50} and LC_{90} values at 12, 24 and 48 hours. These are shown in Table 4.16-4.19.

Table 4.16

The twelve fractions derived from the column chromatography of the hexane extract of the leaves of *Carica papaya* were tested against the larvae of *Aedes vittatus* and fraction 1 gave the lowest LC_{50} and LC_{90} values of 10.7ppm and 24.3ppm.

Table 4.17

The fractions of the hexane leaf extract of *Carica papaya* were tested against the larvae of *Culex quinquefasciatus* and fraction 1 gave the lowest LC_{50} and LC_{90} values of 15.1ppm and 326.3ppm.

Table 4.18

The seven (7) fractions derived from the column chromatography of the ethanol seed extract of *Dacryodes edulis* were tested against the larvae of *Aedes vittatus* and fraction 1 gave the lowest LC_{50} and LC_{90} values of 10.4ppm and 58.3ppm.

Table 4.19

The fractions from the ethanol seed extract of *Dacryodes edulis* were tested against the larvae of *Culex quinquefasciatus* and fraction 1 gave the lowest LC_{50} and LC_{90} values of 10.1ppm and 33.5ppm.

Table 4.16: Larvicidal Activity of Partially Purified Fractions of *Carica papaya* Hexane Leaf Extract (CPHLE) against *Aedes vittatus* at 48 hours.

Aedes vittatus **(CPHLE)**

Fraction	Conc (ppm)	6.25	12.5	25	50	100	Lethal Conc (ppm)	
	Time (hrs)	Percentage Mortality (%)					LC_{50} (UCL – LCL)	LC_{90} (UCL- LCL)
F-1	12	24.0±0.0	48.0±10.5	76.0±4.0	93.3±6.1	100.0±4.0	**10.7** (13.1-8.4)	**24.3** (38.1-18.8)
	24	24.0±0.0	52.0±6.9	76.0±4.0	93.3±6.1	100.0±4.0		
	48	24.0±0.0	52.0±6.9	76.0±4.0	93.3±6.1	100.0±4.0		
F-2	12	-	-	8.0±4.0	12.0±8.0	17.3±6.1	**229.1** (661328.0-77.7)	**1214.0** (1.1ε+14)
	24	4.0±4.0	8.0±4.0	16.0±8.0	21.6±4.6	32.0±4.6		
	48	12.0±4.0	16.0±0.0	28.0±12.8	28.0±4.0	40.0±4.0		
F-3	12	-	4.0±0.0	12±4.0	8.0±4.0	40.0±10.5	**48.8** (84.1-33.9)	**320.1** (1582.0-153.0)
	24	8.0±4.0	12.0±4.0	22.6±6.1	16.0±4.0	60.0±12		
	48	12.0±4.0	17.3±2.3	24.0±0.0	40.0±10.0	80.0±10		
F-4	12	8.0±0.0	8.0±4.0	-	8.0±4.0	16.0±4.0	**132.0** (18992.0-54.6)	**6915.9** (1.6ε+16- 608.1)
	24	16.0±4.0	16.0±4.0	8.0±4.0	16.0±8.0	32.0±8.3		
	48	16.0±4.0	24.0±0.0	28.0±0.0	36.0±4.0	48.1±6.1		
F-5	12	4.0±0.0	4.0±0.0	12.0±4.0	12.0±4.0	12.0±4.0	**188.2** (1284182.0-65.9)	**13196.0** (2.1ε+16-779.9)
	24	12.0±4.0	12.0±4.0	22.6±6.1	24.1±6.1	28.0±6.1		
	48	16.0±4.0	16.0±0.0	32.0±8.0	36.0±8.0	40.0±8.3		
F-6	12	8.0±0.0	12.0±4.0	12.0±4.0	32.0±10.5	80.0±0.0	**18.8** (68.605.0-1.6)	**111.6** (39942865.0-41.6)
	24	25.3±6.1	21.3±8.3	20.0±0.0	68.0±2.3	92.0±0.0		
	48	32.0±4.0	32.0±4.6	40.0±0.0	84.0±4	92.0±0.0		
F-7	12	8.0±0.0	4.0±4.0	8.0±0.0	12.0±4.0	12.0±4.0	**335.9** (12629542834.0-91.2)	**26443.0** (2.3 ε+25-1037.0)
	24	12.0±4.0	8.0±4.0	16±4.0	20.0±8.0	20.0±8.0		
	48	12.0±4.0	16.0±0.0	24.0±4.0	28.0±4.0	36.0±4.0		
F-8	12	8.0±4.0	12.0±4.0	12.0±4	16.0±4.0	12.0±0.0	**58.6** (252.435-32.646)	**1455.0** (6656.54-304.54)
	24	12.0±4.0	25.2±6.1	22.6±4.6	26.6± 6.1	22.6±4.6		
	48	16.0±4.0	32.0±4.6	36.0±8.0	42.0±4.0	58.0±4.0		
F-9	12	8.0±0.0	12.0±4.0	20.0±4.0	16.0± 4.0	17.3±10.0	**51.9** (4630.0-22.5)	**5810.0** (1.9ε+17 – 448.6)
	24	17.3±4.6	24.0±0.0	36.0±8.0	32.0±8.0	34.6±4.6		
	48	22.6±2.3	40.0±10.0	48.0±6.1	48.0±4.6	56.0± 6.1		
F-10	12	16.0±4.0	24.0±8.0	28.0±4.0	12.0±4.0	32.0±8.0	**22.8** (49.1-9.4)	**803.5** (529063.0-187.7)
	24	32.0±8.0	32.0±4.0	48.0±6.1	26.6±8.3	57.3±8.3		
	48	32.0±8.0	44.0±8.0	48.0±6.1	60.0±8.0	72.0±6.1		
F-11	12	8.0±0.0	8.0±4.0	16.0± 4.0	16.0±0.0	20.0±4.0	**75.7** (5362.9-34.4)	**5602.0** (3.6ε+12-494.2)
	24	17.3±4.6	24.0±0.0	36.0±8.0	32.0±8.0	36.0±4.6		
	48	22.6±2.3	32.0±6.1	40.0±0.0	44.0±4.6	52.0± 6.1		
F-12	12	17.3±10.0	24.0±0.0	28.0±4.0	28.0±4.0	32.0±8.0	**44.6** (2230.0-18.6)	**5236.9** (3.2ε+17-418.1)
	24	40.0±6.1	32.0±4.0	32.0±6.1	40.0±6.1	48.0±6.1		
	48	40.0±0.0	40.0±8.3	44.0±0.0	48.0±8.0	60.0±0.0		

F1-F12: Twelve (12) fractions derived from the partial purification of CPHLE (*Carica papaya* hexane leaf extract). Mortality values represent Mean±SD of 3 replicates of r=25

Table 4.17: Larvicidal Activity of Partially Purified Fractions of *Carica papaya* Hexane Leaf Extract (CPHLE) against *Culex quinquefasciatus*at 48 hours.

Culex quinquefasciatus (CPHLE)

Fraction	Conc (ppm)	6.25	12.5	25	50	100	Lethal Conc (ppm)	
	Time (hrs)	Percentage Mortality (%)					LC50 (UCL-LCL)	LC90 (UCL-LCL)
F-1	12	16.0±4.0	21.3±8.3	46.6±6.1	60.0±8.0	80.0±0.0	**15.1** (26.2-5.7)	**326.3** (11796.5-113.8)
	24	20.0±4.0	32.0±4.0	56.0±6.9	64.0±4.0	80.0±0.0		
	48	40.0±0.0	40.0±10.0	60.0±6.9	68.0±4.0	80.0±0.0		
F-2	12	-	4.0±0.0	8.0±4.0	12.0±4.0	21.3±6.1	**112.5** (1076.7-56.3)	**2365.2** (2343362.0-425.1)
	24	4.0±4.0	12.0±4.0	16.0±8.0	22.6±4.6	37.3±4.6		
	48	8.0±4.0	21.3±6.1	21.3±6.1	34.6±6.1	46.6±4.6		
F-3	12	-	4.0±0.0	4.0±0.0	8.0±4.0	24.0±0.0	**196.0** (5636.8-83.4)	**3868.6** (20575867.0-563.7)
	24	4.0±0.0	8.0±4.0	12.0±4.0	16.0±4.0	24.0±0.0		
	48	8.0±0.0	12.0±4.0	16.0±4.0	25.3±6.1	40.0±8.0		
F-4	12	4.0±4.0	4.0±0.0	4.0±4.0	8.0±0.0	12.0±4.0	**108.7** (739.4-57.0)	**1819.0** (533179.0-383.9)
	24	4.0±0.0	12.0±4.0	16.0±8.0	16.0±4.0	26.6±8.3		
	48	4.0±0.0	22.6±4.6	28.0±4.0	36.0±6.1	45.3±4.6		
F-5	12	8.0±4.0	8.0±0.0	12.0±4.0	16.0±8.0	21.3±6.1	**58.4** (239.3-32.8)	**1376.2** (520248.0-297.7)
	24	17.3±8.3	22.6±4.6	28.0±6.1	32.0±4.0	32.0±4.6		
	48	24.0±4.0	33.3±8.3	44.0±4.0	46.6±9.2	52.0±8.0		
F-6	12	-	4.0±4.0	4.0±0.0	4.0±0.0	64.0±4.0	**68.8** (134.3-46.7)	**423.3** (2520.0-191.5)
	24	4.0±4.0	8.0±4.0	8.0±0.0	28.0±6.1	68.0±0.0		
	48	8.0±0.0	12.0±4.0	20.0±0.0	28.0±6.1	72.0±0.0		
F-7	12	4.0±4.0	12.0±4.0	4.0±4.0	4.0±0.0	8.0±4.0	**99.1** (926.1-50.1)	**2408.0** (3802480.0-415.5)
	24	8.0±4.0	16.0±4.0	16.0±4.0	21.6±6.1	25.3±8.3		
	48	12.0±0.0	20.0±4.0	32.0±8.0	40.0±4.0	48.0±0.0		
F-8	12	12.0±4.0	12.0±4.0	16.0±4.0	16.0±4.0	20.0±4.0	**75.7** (5362.0-34.4)	**5602.9** (3.6ε+12)
	24	20.0±8.0	22.6±6.1	32.0±8.0	32.0±8.0	37.3±6.1		
	48	20.0±4.0	32.0±6.1	40.0±6.1	44.0±10.5	52.0±4.0		
F-9	12	8.0±4.0	8.0±4.0	12.0±4.0	8.0±4.0	16.0±4.0	**118.1** (2803.0-54.4)	**3856.3** (120772245.0-501.8)
	24	12.0±4.0	16.0±4.0	22.6±4.6	17.3±6.1	32.0±8.0		
	48	12.0±8.0	24.0±0.0	28.0±4.0	36.0±8.0	48.0±6.1		
F-10	12	8.0±0.0	16.0±4.0	24.0±4.0	18.6±8.3	40.0±4.0	**65.4** (591.1-33.4)	**2568.1** (4990112.0-378.9)
	24	24.0±8.0	25.3±6.1	34.6±6.1	24.0±4.0	48.0±6.1		
	48	28.0±4.0	32.0±6.1	40.0±4.0	48.0±8.0	52.0±6.1		
F-11	12	8.0±0.0	8.0±4.0	16.0± 4.0	16.0±0.0	20.0±4.0	**59.2** (1114.6-28.3)	**3853.6** (326349211145.0-416.0)
	24	17.3±4.6	24.0±0.0	36.0±8.0	32.0±8.0	36.0±4.6		
	48	22.6±2.3	32.0±6.1	40.0±0.0	48.0±4.6	56.0± 6.1		
F-12	12	16.0±0.0	24.0±0.0	28.0±4.0	28.0±4.0	32.0±8.0	**51.6** (230.6-28.0)	**1595.5** (2121830.0-303.7)
	24	32.0±10.8	32.0±4.0	32.0±6.1	40.0±6.1	48.0±6.1		
	48	32.0±6.1	40.0±8.3	40.0±0.0	48.0±8.0	60.0±0.0		

F1-F12: Twelve (12) fractions derived from the partial purification of CPHLE (*Carica papaya* hexane leaf extract). Mortality values represent Mean±SD of 3 replicates of r=25

Table 4.18: Larvicidal Activity of Partially Purified Fractions of *Dacryodes edulis* Ethanol Seed Extract (DEESE) against *Aedes vittatus* at 48 hours.

Aedes vittatus (DEESE)

Fraction	Conc (ppm)	6.25	12.5	25	50	100	Lethal Conc (ppm)	
	Time (hrs)	Percentage Mortality (%)					LC$_{50}$ (UCL-LCL)	LC$_{90}$ (UCL-LCL)
F-1	12	16.0±8.0	20.0±10.5	34.6±6.1	32.0±8.0	38.6±10.0	**10.4**	**58.3**
	24	28.0±4.0	32.0±8.0	68.0±8.0	73.3±4.6	88.0±8.0	(14.9-6.0)	(132.5-37.8)
	48	36.0±4.0	44.0±4.0	90.6±4.6	88.0±4.0	92.0±4.0		
F-2	12	8.0±4.0	8.0±0.0	8.0±0.0	22.6±4.6	48.0±6.1	**63.1**	**1338.8**
	24	17.3±4.6	16.0±4.0	17.3±6.1	45.3±4.6	52.0±8.0	(251.5-35.6)	(275237.0-304.0)
	48	21.3±4.6	20.0±4.0	28.0±4.0	60.0±0.0	52.0±8.0		
F-3	12	8.0±4.0	20.0±0.0	8.0±4.0	12.0±4.0	24.0±4.0	**37.8**	**1828.1**
	24	21.3±6.1	28.0±4.0	17.3±4.6	44.0±8.0	45.3±4.6	(165.1-18.4)	(47527425.0-291.4)
	48	36.0±0.0	36.0±4.0	46.6±4.6	52.0±6.1	64.0±4.0		
F-4	12	12.0±4.0	17.3±6.1	18.6±8.3	22.6±4.6	24.0±4.0	**51.7**	**2267.6**
	24	20.0±0.0	32.0±4.0	36.0±4.0	40.0±8.0	48.0±8.0	(346.8-26.5)	(58054822.0-341.5)
	48	20.0±0.0	32.0±4.0	40.0±0.0	52.0±0.0	56.0±6.1		
F-5	12	12.0±4.0	8.0±4.0	16.0±4.0	16.0±4.0	18.6±8.3	**23.2**	**424.262**
	24	26.6±4.6	17.3±10	28.0±4.6	32.0±8.0	37.3±6.1	(41.7-12.2)	(12508.0-144.4)
	48	28.0±4.0	37.3±6.1	56.0±6.1	64.0±12.0	72.0±8.0		
F-6	12	-	8.0±4.0	-	4.0±4.0	12.0±4.0	**106.0**	**746.2**
	24	-	16.0±8.0	12.0±0.0	16.0±4.0	28.0±6.1	(316.1-65.2)	(11831.0-268.0)
	48	-	20.0±4.0	12.0±0.0	25.3±6.1	45.3±4.6		
F-7	12	4.0±0.0	16.0±4.0	8.0±4.0	12.0±4.0	16.0±4.0	**71.3**	**1558.0**
	24	8.0±4.0	25.3±6.1	16±4.0	28.0±6.1	34.6±6.1	(350.4-39.3)	(562781.0-328.7)
	48	12.0±4.0	25.3±6.1	34.6±4.6	40.0±6.1	56.0±8.0		

F1-F7: Seven (7) fractions derived from the partial purification of DEESE (*Dacryodes edulis*seed ethanol extract).
Mortality values represent Mean±SD of 3 replicates of r=25

Table 4.19: Larvicidal Activity of Partially Purified Fractions of *Dacryodes edulis* Ethanol Seed Extract (DEESE) against *Culex quinquefasciatus* at 48 hours.

Culex quinquefasciatus (DEESE)

Fraction	Conc (ppm)	6.25	12.5	25	50	100	Lethal Conc (ppm)	
	Time (hrs)	Percentage Mortality (%)					LC_{50} (UCL-LCL)	LC_{90} (UCL-LCL)
F-1	12	12.0±0.0	16.0±6.9	48.0±24.0	60.0±10.5	60.0±13.8	**10.1** (21.3-0.5)	**33.5** (7191.0-16.9)
	24	24.0±4.0	32.0±14.4	72.0±13.8	92.0±10.5	96.0±6.9		
	48	28.0±0.0	52.0±10.5	96.0±4.0	96.0±4.0	96.0±6.9		
F-2	12	4.0±0.0	4.0±4.0	4.0±4.0	8.0±4.0	4.0±0.0	**75.2** (317.5-42.5)	**1335.0** (191529.0-316.6)
	24	8.0±4.0	12.0±4.0	8.0±0.0	32.0 ±12.0	8.0±0.0		
	48	12.0±6.9	16.0±8.0	40.0±10.5	48.0±10.5	48.0±0.0		
F-3	12	4.0±0.0	16.0±8.0	12.0±6.9	16.0±0.0	24.0±13.8	**35.2** (117.4-17.4)	**1425.0** (5720627.0-263.6)
	24	16.0±4.0	24.0±8.0	24.0±4	40.0±10.0	40.0±4.0		
	48	24.0±13.8	32.0±0.0	56.0±10.5	60.0±8.0	56.0±8.0		
F-4	12	-	8.0±0.0	20.0±10.5	12.0±4.6	20.0±6.9	**36.4** (78.2-21.6)	**627.1** (24806.0-194.1)
	24	4.0±0.0	16.0±4.0	40.0±6..9	36.0±6.9	36.0±4.0		
	48	16.0±4.0	32.0±4.0	52.0±13.8	60.0±4.0	60.0±4.0		
F-5	12	4.0±0.0	8.0±4.0	16.0±0.0	-	4.0±0.0	**77.3** (587.6-40.5)	**2190.2** (5100712.0-377.1)
	24	8.0±4.0	12.0±4.0	16.0±0.0	16.0±4.0	12.0±4.0		
	48	12.0±4.0	20.0±4.0	48.0±6.9	48.0±8.0	44.0±10.5		
F-6	12	-	4.0±4.0	16.0±4.0	12.0±4	24.0±0.0	**93.5** (626.6-49.3)	**1920.0** (95521.0-380.1)
	24	4.0±0.0	8.0±4.0	24.0±6.9	28.0±6.9	48.0±10.5		
	48	12.0±0.0	12±0	40.0±10.5	44.0±4.0	56.0±6.9		
F-7	12	-	4.0±0.0	-	8.0±4.0	4.0±0.0	**317.5** (146763.0-108.3)	**7280.8** (13825822165.0-715.8)
	24	-	12.0±0.0	-	16.0±8.0	8.0±4.0		
	48	-	16.0±0.0	16.0±0.0	24.0±4.0	28.0±0.0		

F1-F7: Seven (7) fractions derived from the partial purification of DEESE (*Dacryodes edulis* ethanol seed extract).
Mortality values represent Mean±SD of 3 replicates of r=25

4.4 Phytochemical Analysis

Table 4.20

Phytochemical analysis of fraction 1 (f1) of the hexane extract of leaf of *Carica papaya* and fraction 1 (f1) of of the ethanol extract of the seed of *Dacryodes edulis*

Phytochemical analysis was carried out on the fraction 1 (f1) of the hexane extract of the leaf of *Carica papaya* and fraction 1(f1) of the ethanol extract of the seed of *Dacryodes edulis.*

The hexane extract of the leaf of *Carica papaya* showed presence of carbohydrates, cardiac glycosides and unsaturated steroids while the ethanol extract of the seed of *Dacryodes edulis* showed the precence of carbohydrates, cardiac glycosides, unsaturated and steroids, tannins and alkaloids.

Table 4.20: Phytochemical analysis of fraction 1 (f1) of the hexane extract leaves of *Carica papaya* and fraction 1(f1) of the ethanol extract of seeds of *Dacryodes edulis*

Test	**Fraction 1 (f1) *Carica papaya***	**Fraction 1 (f1) *Dacryodes edulis***
Carbohydrate	+ve	+ve
Saponins	-ve	-ve
Cardiac glycosides	+ve	+ve
Unsaturated Steroids	+ve	+ve
Unsaturated Triterpenes	+ve	+ve
Tannins Test	-ve	+ve
Alkaloids	-ve	+ve
Flavonoids	-ve	-ve

+ve: Present

-ve: Absent

4.5 Mode of Action

4.5.1 Growth inhibitory activity

Table 4.21, 4.22, 4.23 and 4.24 show the growth inhibitory activity of the most active larvicidal fraction (f1) of *Carica papaya* and the fraction (f1) of *Dacryodes edulis*. Adult emergence was completed between twelve and thirteen (12-13) days in first (1st) instar, ten (10) days in the second (2nd) instar, between five and seven (5-7) days in the early third (3rd) instar and four-six (4-6) days in the late third and early fourth (3rd-4th) instar in the control groups.

Table 4.21: Growth inhibition activity of 10ppm of fraction1 of *Carica papaya* hexane leaf extract (CPHLE-f1) on the 1st-4th instars of *Aedes vittatus.*

Fraction 1 (f1) of *Carica papaya* hexane leaf extract gave mortality of 12%, 16% and 26.67% at 12, 24 and 48 hours respectively at 10ppm (LC_{50} of CPHLE-f1) against the first (1st) instar larvae of *Aedes vittatus*. Complete emergence of control occurred on day thirteen (13) and growth inhibition was 14.84%. Mortality of (second) 2nd instar mortality was 36% as at 48 hours and complete emergence occurred on day ten (10) with 43.14% growth inhibition. The early third (3rd)instar of the larvae of *Aedes vittatus* gave mortality values of 20%, 22% and 24% at 12, 24 and 48 hrs with growth inhibition of 48%. Complete emergence of control was on day six (6).The late third and early fourth (3rd and 4th) instars gave mortality of values of 16%, 24% and 40% at 12, 24 and 48 hrs and complete emergence occurred on day four (4) with growth inhibition of 60%.

Table 4.22: Growth inhibition activity of 15ppm of fraction 1of *Carica papaya* hexane leaf extract (CPHLE-f1) on the 1st-4th instars of *Culex quinquefasciatus*

Fraction 1 (f1) of the *Carica papaya* hexane leaf extract gave mortality of 8%, 12% and 20% at 12, 24 and 48 hours respectively at15ppm (LC_{50} of CPHLE-f1) against the first (1st) instar larvae of *Culex quinquefasciatus*. Growth inhibition was 23.61% with complete emergence occurring on day twelve (12). Second (2nd) instar mortality was 4%, 16% and 16% at 12, 24 and 48 hours and complete emergence on day nine (9) with 52.32% growth inhibition. The early third (3rd)instar of the larvae of *Culex quinquefasciatus* gave mortality values of 4%, 20% and 20% with complete emergence on day five (5) and growth inhibition of 52.66% while the late third and early fourth (3rd and 4th) instars gave mortality of values of 24%, 36% and 44% with growth inhibition of 66.6% and complete emergence was on day three (3).

Table 4.23: Growth inhibition activity of 10ppm of fraction 1 (f1) of *Dacryodes edulis* ethanol seed extract (DEESE-f1) on the 1st-4th instars of *Aedes vittatus*

Fraction 1 (f1) of *Dacryodes edulis* ethanol seedextract gave mortality of 4%, 4% and 12% at 12, 24 and 48 hours respectively at 10ppm (LC_{50} of DEESE-f1) against the first (1st) instar larvae of *Aedes vittatus*, however no growth inhibition occurred. Mortality of (second) 2nd instar was 8% as at 48 hours with 0.76% growth inhibition after complete emergence on day nine (9). The early third (3rd) instar of the larvae of *Aedes vittatus* gave mortality values of 16%, 24% and 28% with growth inhibition of 44.43%. Complete emergence occurred on day five (5) while the late third and early fourth (3rd and 4th)

instars gave mortality of values of 16%, 24 and 36% with growth inhibition of 46.67% and complete emergence on day three (3).

Table 4.24: Growth inhibition activity of 10ppm of fraction 1 of *Dacryodes edulis* ethanol seed extract (DEESE-f1) on the 1st-4th instars of *Culex quinquefasciatus*

Fraction (f1) of the *Dacryodes edulis* ethanol seed extract gave mortality 12% at 48 hours at 10ppm (LC_{50} of DEESE-f1) against the first (1st) instar larvae of *Culex quinquefasciatus*. Growth inhibition was 4.31% and complete emergence was on day twelve (12). Motality of (second) 2nd instar was 8%, 8% and 12 % at 12, 24 and 48 hours but there was no growth inhibition. The early third (3rd)instar of the larvae of *Culex quinquefasciatus* gave mortality values of 12%, 18% and 20% with growth inhibition of 34.18%. The late third and early fourth (3rd and 4th) instars gave mortality of values of 18, 36 and 48% and complete emergence occurred on day four (4) with growth inhibition of 65.78%.

Table 4.21: Growth Inhibtion of Instar 1-4 of the larvae of *Aedes vittatus* by 10ppm (1mg/dl) (LC_{50}) of fraction1 of *Carica papaya* hexane leaf extract (CPHLE-f1).

Instars	(%) Percentage Mortality (Mean±SD)				No (Percentage (%) Survival Mean±SD)		No (Percentage (%) Emergence Mean±SD)		Percentage (%) Inhibition
	Time (hrs)	**DCE**	**Test**	**Control**	**Test**	**Control**	**Test**	**Control**	
1st	12	13	12.00±4.00	-	55(73.34±18.47)	65(86.67±2.30)	48(64.00±13.85)	65(86.67±2.30)	14.84
	24		16.00±10.58	-			Corrected **73.80%**		
	48		26.67±4.60	8.00±4.60					
2nd	12	10	12.00±6.90	-	40(53.34±6.10)	68(90.60±9.86)	35(46.67±6.10)	68(90.60±9.86)	43.14
	24		12.00±6.90	-			Corrected **51.15%**		
	48		36.00±10.58	-					
3rd	12	6	20.00±8.00	-	42(56.00±18.30)	75(100.00±0.00)	39(52.00±6.92)	75(100.00±0.00)	48.00
	24		22.00±8.32	-					
	48		24.00±6.92	-					
4th	12	4	16.00±0.00	-	39(52.00±14.40)	75(100.00±0.00)	30(40.00±0.00)	75(100.00±0.00)	60.00
	24		24.00±6.92	-					
	48		40.00±4.00	-					

Mortality values represent Mean±SD of 3 replicates of r=25
DCE- Day of complete emergence of control.
Control: larvae subjected to declorinated water+acetone+tween 80 only
Test: 1st to 4th Instar larve subjected to 10ppm of fraction f1+acetone+tween 80 in declorinated water

Table 4.22: Growth Inhibtion of Instar 1-4 of the larvae of *Culex quinquefasciatus* by 15ppm (1.5mg/dl) (LC_{50}) of fraction 1 of *Carica papaya* hexane leaf extract (CPHLE-f1).

Instars	**Percentage Mortality (%)** (Mean±SD)				**No (Percentage (%) Survival** Mean±SD)		**No (Percentage (%) Emergence** Mean±SD)		**Percentage (%) Inhibition**
	Time (hrs)	**DCM**	**Test**	**Control**	**Test**	**Control**	**Test**	**Control**	
1st	12	12	8.00±4.00	-	56(64.00±16.16)	70(86.67±2.30)	50(66.66±16.60)	70(93.37±2.30)	23.61
	24		12.00±6.92	-			Corrected **71.32%**		
	48		20.00±8.00	-					
2nd	12	9	4.00±0.00	-	42(46.67±10.50)	71(90.60±2.30)	32(42.67±4.60)	71(94.60±2.30)	52.32
	24		16.00±4.00	4.00±4.00			Corrected **45.10%**		
	48		16.00±4.00	4.00±4.00					
3rd	12	5	4.00±0.00	-	42(52.00±6.90)	74(98.60±2.30)	35(46.67±4.60)	74(98.60±2.30)	52.66
	24		20.00±8.00	-					
	48		20.00±8.00	-					
4th	12	3	24.00±10.50	-	37(49.30±8.30)	75(100.00±0.00)	25(33.34±6.10)	75(100.00±0.00)	66.66
	24		36.00±6.92	-					
	48		44.00±4.00	-					

Mortality values represent Mean±SD of 3 replicatates of r=25
DCE- Day of complete emergence of control
Control: larvae subjected to declorinated water+acetone+tween 80 only
Test: 1st to 4th Instar larve subjected to 10ppm of fraction f1+acetone+tween 80 in declorinated water

Table 4.23: Growth Inhibtion of Instar 1-4 of the larvae of *Aedes vittatus* by 10ppm (1mg/dl) (LC_{50}) of fraction 1of *Dacryodes edulis* ethanol seed extract (DEESE-f1).

Instars	Percentage Mortality (%) (Mean±SD)				No (Percentage (%) Survival Mean±SD)		No (Percentage (%) Emergence Mean±SD)		Percentage (%) Inhibition
	Time (hrs)	**DCE**	**Test**	**Control**	**Test**	**Control**	**Test**	**Control**	
1st	12	13	4.00±0.00	-	60(80.00±8.00)	64(85.30±2.30)	58(77.34±8.30)	64(85.3±2.30)	-
	24		4.00±0.00	-			Corrected **90.66%**		
	48		12.00±4.00	8.00±4.00					
2nd	12	9	8.00±4.00	-	65(86.67±6.10)	69(92.00±4.00)	63(84.00±8.00)	69(92.00±4.00)	0.76
	24		8.00±4.00	-			Corrected **91.30%**		
	48		8.00±4.00	-					
3rd	12	5	16.00±6.90	-	50(66.67±16.60)	72(96.00±6.90)	40(53.34±11.54)	72(96.00±6.90)	44.43
	24		24.00±13.80	-					
	48		28.00 ±14.40	-					
4th	12	3	16.00±10.58	-	48(64.00±6.90)	75(100.00±0.00)	40(53.34±14.00)	75(100.00±0.00)	46.67
	24		24.00±6.92	-					
	48		36.00±4.61	-					

Mortality values represent Mean±SD of 3 replicates of r=25
DCE- Day of complete emergence of control
Control: larvae subjected to declorinated water+acetone+tween 80 only
Test: 1st to 4th Instar larve subjected to 10ppm of fraction f1+acetone+tween 80 in declorinated water

Table 4.24: Growth Inhibtion of Instar 1-4 of the larvae of *Culex quinquefaciatus* by 10ppm (1mg/dl) (LC_{50})of fraction 1of *Dacryodes edulis* ethanol seed extract (DEESE-f1).

Instars	Percentage Mortality (%) (Mean±SD)				No (Percentage (%) Survival Mean±SD)		No (Percentage (%) Emergence Mean±SD)		Percentage (%) Inhibition
	Time (hrs)	DCE	Test	Control	Test	Control	Test	Control	
1st	12	12	-	-	65(86.67±12.80)	68(90.67±8.30)	59(78.67±10.00)	68(90.67±8.30)	4.31
	24		12.00±0.00	-			Corrected **86.76**		
	48		12.00±0.00	16.00±6.90					
2nd	12	9	8.00±4.00	-	68(90.67±10.00)	70(93.34±2.30)	67(89.34±8.30)	70(93.34±2.30)	-
	24		8.00±4.00	-			Corrected **95.77**		
	48		12.00±4.00	-					
3rd	12	7	12.00±4.00	-	55(73.34±6.10)	70(93.34±6.10)	43(57.34±6.10)	70(93.34±6.10)	34.18
	24		18.00±2.30	-			Corrected **61.43**		
	48		20.00±0.00	-					
4th	12	4	18.00±4.00	-	34(45.34±4.61)	71(94.96±6.10)	23(30.67±4.60)	71(94.67±6.10)	65.78
	24		36.00±10.50	-			Corrected **32.39**		
	48		48.00±4.00	-					

Mortality values represent Mean±SD of 3 replicatates of r=25
DCE- Day of complete emergence of control
Control: larvae subjected to declorinated water+acetone+tween 80 only
Test: 1st to 4th Instar larve subjected to 10ppm of fraction f1+acetone+tween 80 in declorinated water

4.5.2 Acetylcholinesterase inhibition assay

Effect of concentrations of 6.25 (.625mg/dl), 12.5 (1.25mg/dl), 25 (2.5mg/dl), 50 (5.0mg/dl) and 100ppm (10mg/dl) on acetylcholinesterase in the late third and early fourth instar of the larvae of *Aedes vittatus* and *Culex quinquefasciatus* was evaluated and the results are shown in figures 4.3 and 4.4.

Figure 4.1

Inhibition at 6.25 (.625mg/dl), 12.5 (1.25mg/dl), 25 (2.5mg/dl), 50 (5.0mg/dl) and 100ppm (10mg/dl) of fraction 1 of *Carica papaya* hexane leaf extract (CPHLE-f1) was 11.44, 13.06, 19.65, 26.94 and 31.02% against the late third and early fourth instars of the larvae of *Aedes vittatus* and 4.33, 4.55, 16.80, 23.09 and 31.26% against *Culex quinquefasciatus.*

Figure 4.2

Inhibition at 6.25 (.625mg/dl), 12.5 (1.25mg/dl), 25 (2.5mg/dl), 50 (5.0mg/dl) and 100ppm (10mg/dl) of fraction 1 of *Dacryodes edulis* ethanol seed extract (DEESE-f1) was 11.44, 13.06, 19.65, 26.94 and 31.02% against the late third and early fourth instar of the larvae of *Aedes vittatus* and 4.33, 4.55, 16.80, 23.09 and 31.26% against *Culex quinquefasciatus.*

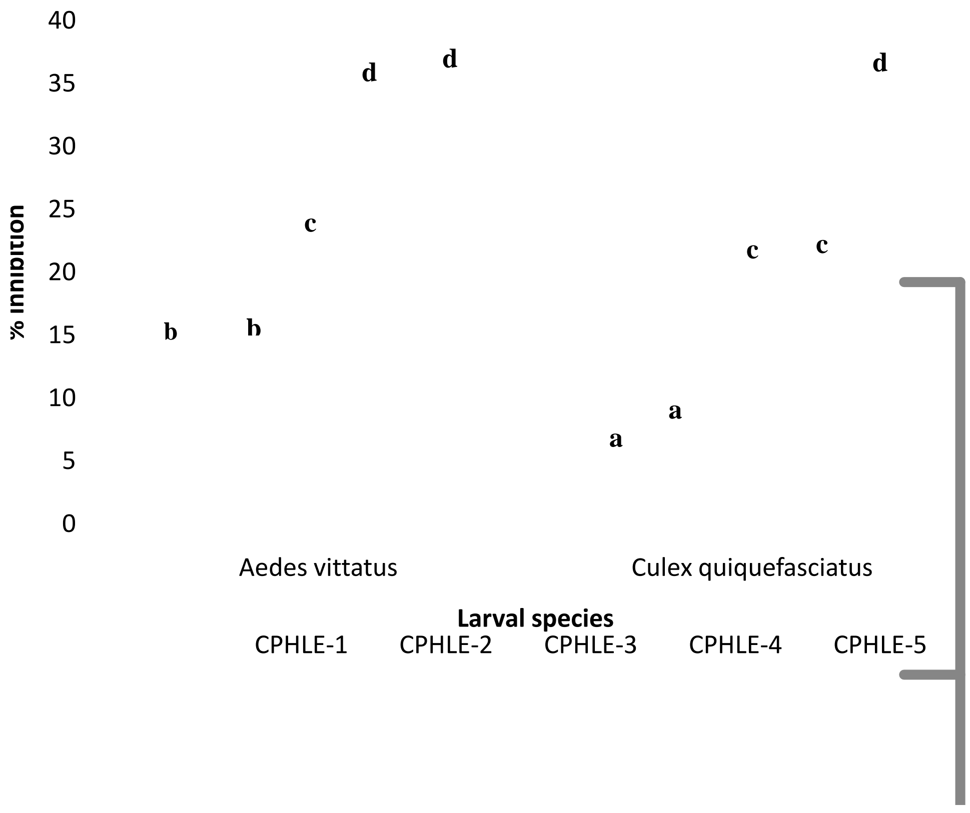

Figure 4.1:Acetylcholinesterase inhibition in *Aedes vittatus* and *Culex quinquefasciatus* by different concentrations of *Carica papaya* hexane leaf extract fraction 1

CPHLE-1-5 : 6.25 (.625mg/dl), 12.5 (1.25mg/dl), 25 (2.5mg/dl), 50 (5.0mg/dl) and 100ppm (10mg/dl) of fraction 1 (f1) of *Carica papaya* hexane leaf extract

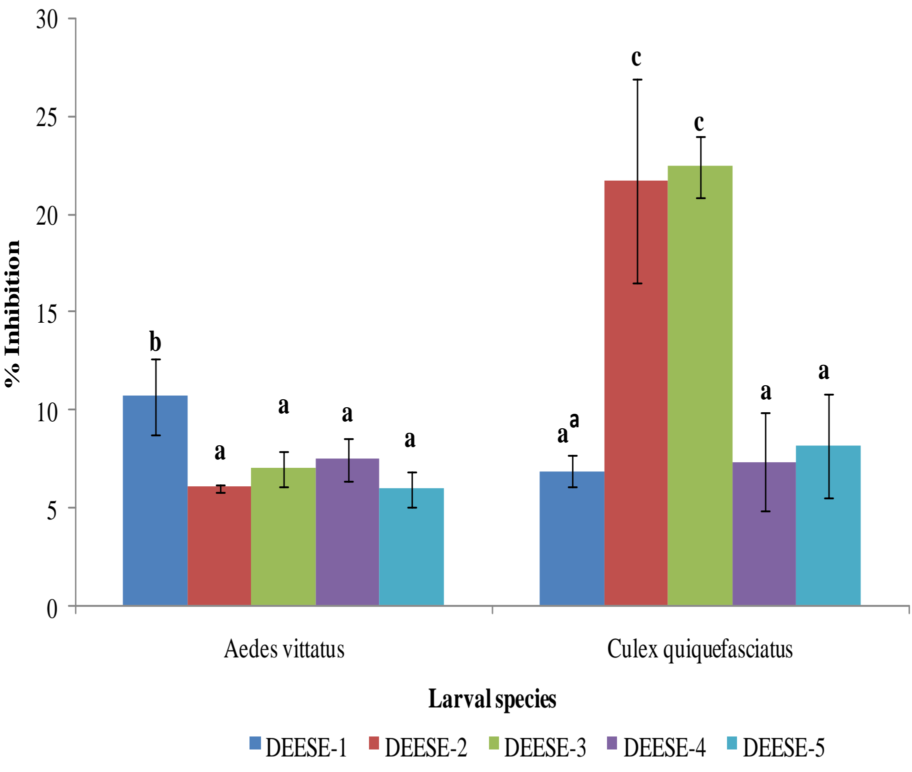

Figure 4.2: Acetylcholinesterase inhibition in *Aedes vittatus* and *Culex quinquefasciatus* by different concentrations of *Dacryodes edulis* ethanol seed extract fraction 1

DEESE-1-5: 6.25 (.625mg/dl), 12.5 (1.25mg/dl), 25 (2.5mg/dl), 50 (5.0mg/dl) and 100ppm (10mg/dl) of fraction 1 (f1) of *Dacryodes edulis* ethanol seed extract

4.6 Effect on a Non-Target Organism of the same habitat

Table 4.25

Effect of concentration at which 50% mortality was achieved of fraction 1 of *Carica papaya* hexane leaf extract and fraction 1 of *Dacryodes edulis* ethanol seed extract on *Poecelia reticulata* (guppy fish)

The effect of 25ppm (2.5mg/dl) of the fraction 1 (f1) of *Carica papaya* hexane leaf extractand 15ppm (1.5mg/dl) of fraction 1 (f1) of *Dacryodes edulis* ethanol seed extract on *Poecelia reticulata* is shown in table 4.25. The readings were taken at 24 hours and 48 hours. The percentage mortality observed at 24 hours and 48 hours when treated with *Carica papaya* were 6.67% and 7.70% respectively while the percentage mortality observed when treated with *Dacryodes edulis* at 24 hours and 48 hours was 4.44%. Values showed no significant difference ($p>0.05$) when compared with controls.

Table 4.26

Effect of LC_{50} and LC_{90} values of fraction 1 of *Carica papaya* hexane leaf extract on *Poecelia reticulata* (guppy fish)

The effect of LC_{50} and LC_{90} values of the test fractions were carried out and shown in Table 4.26. The fish were subjected to 15ppm (1.5mg/dl) and 326ppm (32.6mg/dl) of CPHLE-f1 and 10ppm (1mg/dl) and 58ppm (5.8mg/dl) of DEESE-f1 in the same manner as the test above. The results mimicked the results of Table 4.25 above with no significant difference between the tests and negative controls.

Table 4.25: Effect of 25ppm (2.5mg/dl) of fractions 1 of *Carica papaya* hexane leaf extract and the *Dacryodes edulis* ethanol seed extract on *Poecelia reticulata* (guppy fish)

	Fraction	Conc (ppm)	No of fish exposed	No (Mean±SD) of dead fish per exposure time		Percentage Mortality (%) (mean±SD) per exposure time	
				24hr	48hr	24hr	48hr
Test 1	CPHLE (f1)	25	90	6(2.00±1.00)	7(2.33±1.15)	(6.67±3.30)[a]	(7.77±3.85)[a]
Control	-		90	8(2.60±0.57)	8(2.60±0.57)	(8.88±1.93)[a]	(8.88±1.93)[a]
Test 11	DEESE(f1)	25	90	4(1.33±0.57)	4(1.33±0.57)	(4.44±1.92)[a]	(4.44±1.92)[a]
Control	-		90	6(2.00±1.73)	6(2.00±1.73)	(6.66±5.77)[a]	(6.66±5.77)[a]

Test 1: 30 fish subjected 25ppm (2.5mg/dl) of CPHLE- f1(fraction f1 of the *Carica papaya*hexane leaf extract) at 24 and 48 hours
Test 11: 30 fish subjected 25ppm (2.5mg/dl) of DEESE- f1 (fraction f1 of *Dacryodes edulis* ethanol seed extract) at 24 and 48 hours
Controls: 30 fish subjected to de-chlorinated tap water only
All tests were run in triplicate. Values with different subscripts are significantly ($p<0.05$) different.

Table 4.26: Effects of 15ppm (1.5mg/dl) and 326ppm (32.6mg/dl) (LC_{50} and LC_{90}) of fraction1 (f1) *Carica papaya*hexane leaf extract and 10ppm (1mg/dl) and 58ppm (5.8mg/dl) (LC_{50} and LC_{90}) of fraction 1 (f1) of *Dacryodes edulis* ethanol seed extract on *Poecelia reticulata* (guppy fish)

Test	Fraction	Conc (ppm)	No of fish exposed	No (Mean±SD) of dead fish per exposure time		Percentage mortality (%) (mean±SD) per exposure time	
				24hr	48hr	24hr	48hr
Test I	CPHLE (f1)	15	90	6(2.00±1.00)	7(2.33±1.15)	(6.67±3.30) [a]	(7.77±3.85) [a]
Test II	CPHLE (f1)	326	90	4(1.33±0.57)	4(1.33±0.57)	(4.44±1.92) [a]	(4.44±1.92) [a]
Test III	DEESE (f1)	10	90	8(2.60±0.57)	8(2.60±0.57)	(8.88±1.93) [a]	(8.88±1.93)[a]
Test IV	DEESE (f1)	58	90	4(1.33±0.57)	4(1.33±0.57)	(4.44±1.92) [a]	(4.44±1.92) [a]
Control		-	90	6(2.00±1.73)	6(2.00±1.73)	(6.66±5.77) [a]	(6.66±5.77) [a]

Test I and II : 30 fish subjected 15ppm (1.5mg/dl) and 326ppm (32.6mg/dl) (LC_{50} and LC_{90}) of CPHLE-f1 (fraction f1 of the *Carica papaya hexane* leaf extract) at 24 and 48 hours
Test III and IV : 30 fish subjected 10ppm (1mg/dl) and 58ppm (5.8mg/dl) (LC_{50} and LC_{90}) of DEESE- f1 (fraction f1 of *Dacryodes edulis* ethanol seed extract) at 24 and 48 hours
Control: 30 fish subjected to de-chlorinated tap water only
All tests were run in triplicate. Values with different subscripts are significantly ($p<0.05$) different.

4.7 Structural Analysis

4.7.1 Characterization of the larvicidal components in the active fraction by Fourier Transform Infra-red Spectroscopy

Tables 4.27-4.28

Characterization of the active larvicidal fraction (f1) of CPHLE and (f1) of DEESE was carried out using FTIR to determine the functional groups in the fractions. The spectra revealed the presence of seven (7) peaks for CPHLE-f1 and eight (8) peaks for DEESE-f1 as shown in Tables 4.27 and 4.28.

In Table 4.27, the peak at 3395.6 indicates the prescence of O-H (alcohol), the peaks between 2922.2 and 2855.1 shows the presence of C-H stretch (alkane/alkyl). The peak around 1714.6 suggest the presence of C=O (aldehydes). The peak at around 1457.4 indicated C-H bend (alkane). The peaks 1375.4 and 775.3 suggest the presence of a N-O (a nitro compound) and C=C (alkene).

Table 4.28 shows peaks 2922.2 – 2855.1 which indicate the presence of C-H (alkane/alkyl). Peak 1736.9 suggest the presence of C=O (aldehyde). The peak at around 1375.4 indicates the presence of N-O (nitro compound) while the peaks at 1162.9 and 1095.8 suggest the presence of C-O (acyl/phenyl and alkoxy) respectively. The peaks at 972.8 and 723.1 indicate the presence of C=C (alkene).

Table 4.27: Fourier Transform Infrared Spectroscopy of larvicidal fraction f1 of *Carica papaya* hexane leaf extract (CPHLE-f1).

Peak s/n	Absorption peak (cm^{-1})	Intensity	Bond	Functional group
1.	3395.6	83.335	O-H	Alcohol
2.	2922.2	69.768	C-H	Alkane/Alkyl
3.	2855.1	80.581	C-H	Alkane/Alkyl
4.	1714.6	70.777	C=O	Carbonyl
5.	1457.4	75.718	C-H bend	Alkane
6.	1375.4	69.005	N-O	Nitro compound
7.	775.3	66.429	C=C	Alkene

CPHLE-f1: *Carica papaya* hexane leaf extract larvicidal fraction 1

Table 4.28: Fourier Transform Infrared Spectroscopy of larvicidal fraction f1 of *Dacryodes edulis* ethanol seed extract (DEESE-f1).

Peak s/n	Absorption peak (cm^{-1})	Intensity	Bond	Functional group
1.	2922.2	55.694	C-H	Alkane/Alkyl
2.	2855.1	67.333	C-H	Alkane/Alkyl
3.	1736.9	56.769	C=O	Aldehyde
4.	1375.4	69.005	N-O	Nitro compound
5.	1162.9	61.635	C-O	Acyl/Phenyl
6.	1095.8	71.658	C-O	Alkoxy
7.	972.8	81.672	C=C	Trans alkene
8.	723.1	81.606	C=C	Alkene

DEESE-f1: ***Dacryodes edulis*** **ethanol seed extract larvicidal fraction 1**

4.7.2 Analysis of the components in the larvicidal active fractions of *Carica papaya* hexane leaf extract and *Dacryodes edulis* ethanol seed extract by Gas Chromatography-Mass Spectroscopy

Tables 4.29-4.30

Analysis of the compounds present in the active larvicidal fraction (f1) of CPHLE and (f1) of DEESE was carried out using GC-MS. The spectra revealed the presence of eleven (11) peaks for CPHLE-f1 and nine (9) peaks for DEESE-f1 as shown in Tables 4.29 and 4.30. The proposed active principles with their retention time (RT), compound name, structure and peak area are shown in both tables.

In Table 4.29, the results showed Cyclohexanone 18.93%; 8-Octadecanol 2.90%; 2-Myristynoyl pantetheine 2.55%; Tetradecane 2,6, 10 trimethyl 4.03%, 3-dodecene 3.64%, 1-Hexadecanol 2 methyl 2.45%, 1- Dodecanol 18.44%, Phenol, 3,5 bis-(1,1-dimethylethyl) 8.16%, 17-Pentatriacotene 3.68%, Dodecyl acrylate 31.84% and 17-Pentatriacotene 3.39%.

In Table 4.30, the results showed Cyclohexanone 5.63%; Tetradecane 2,6, 10 trimethyl 4.25%, Tetradecane 2,6, 10 trimethyl 6.60%, 3-Dodecene 5.85%; 1-Decanol 28.10%; Phenol, 3,5 bis-(1,1-dimethylethyl) 4.97%; 17-Pentatriacotene 4.17%, 17-Pentatriacotene 8.79% and Hexadecanol, 2 methyl 31.64%.

Table 4.29: Proposed compounds present in the bioactive fraction (f1) of *Carica papaya* hexane leaf extract by GC-MS.

Peak	RT	PA%	Compound	Structure
1.	8.975	18.93	Cyclohexanone	
2.	9.423	2.90	8-Octadecanol	
3.	14.215	2.55	2-Myristynoyl pantetheine	
4.	18.425	4.03	Tetradecane 2,6, 10 trimethyl	
5.	20.994	3.64	3-dodecene	
6.	27.812	2.45	1- Hexadecanol, 2 methyl	
7.	30.334	18.44	1- Dodecanol	
8.	31.465	8.16	Phenol 3, 5-bis(1,1-dimethylethyl)	
9.	36.453	3.68	17-Pentatriacotene	
10.	36.838	31.84	Dodecyl acrylate	
11.	38.056	3.39	17-Pentatriacotene	

RT- Retention time
PA-Peak area

Table 4.30: Proposed compounds present in the bioactive fraction (f1) of *Dacryodes edulis* ethanol seed extract by GC-MS.

Peak	RT	PA%	Compound	Structure
1.	9.015	5.63	Cyclohexanone	
2	18.072	4.25	Tetradecane, 2, 6, 10 trimethyl	
3	18.449	6.60	Tetradecane, 2, 6, 10 trimethyl	
1.	21.025	5.85	3-Dodecene	
2.	30.373	28.10	1-Decanol	HO
3.	31.489	4.97	Phenol, 2,4-bis (1,1-dimethylethyl)	OH
7	33.916	4.17	17-Pentatriacotene	
8	36.492	8.79	17-Pentatriacotene	
9.	36.862	31.64	Hexadecanol, 2 methyl	HO

RT- Retention time
PA- Peak area

4.8 Larvicidal Testing (Assay IV)

4.8.1 Larvicidal activity of sub-fractions of CPHLE-f1 and DEESE-f1

Tables 4.31 and 4.32:

Table 4.31 shows the five (5) sub- fractions from CPHLE- f1 were tested for larvicidal activity against the late third and ealy third instar of *Aedes vittatus*. Sub- fraction 3 provided the highest larvicidal activity with LC_{50} value of 9.71ppm at 24 hours while table 4.32 shows the four (4) sub- fractions of DEESE-f1 were tested for larvicidal activity and the LC_{50} values were 31.38, 33.45, 94.43 and 44.73ppm for sub- fractions 1, 2, 3 and 4 respectively.

Table 4.31: Larvicidal activity of sub-fractions of *Carica papaya* hexane leaf extract fraction 1 (CPHLE-f1)

	Conc (ppm)	**12.5**	**25**	**Lethal Conc (ppm)**	**Regression equation**
Sub-fractions	**Time (hrs)**	**Percentage Mortality (%)**		**LC_{50}**	
SF-1	12	42.66±8.30	48.00±4.00	14.33	y = 0.531x+4.346
	24	49.30±4.00	54.66±6.10		
SF-2	12	38.66±10.00	41.3±8.30	30.02	y = 0.398x+4.412
	24	44.00±6.90	49.30±2.30		
SF-3	12	57.30±4.60	64.00±6.90	9.71	y = 1.627x+3.394
	24	57.30±4.60	74.60±4.60		
SF-4	12	26.66±6.10	29.30±6.10	50.00	y = 0.598x+3.984
	24	36.00±6.90	42.66±6.10		
SF-5	12	40.00±4.00	46.66±6.10	22.76	y = 0.697x+4.054
	24	42.60±4.60	50.60±4.60		

SF1-SF5: Five (5) sub fractions derived from the purification of CPHLE-f1 (*Carica papaya* hexane leaf extract fraction 1).
Mortality values represent Mean±SD of 3 replicates of r=25

Table 4.32: Larvicidal activity of sub-fractions of *Dacryodes edulis* ethanol seed extract fraction 1 (DEESE-f1)

	Conc (ppm)	**12.5**	**25**	**Lethal Conc (ppm)**	**Regression equation**
Sub-fractions	**Time (hrs)**	**Percentage Mortality (%)**		**LC_{50}**	
SF-1	12	24.00±8.00	42.60±8.30	31.38	y = 1.528x+2.713
	24	26.66±6.10	44.66±10.58		
SF-2	12	21.30±6.10	38.66±6.10	33.45	y = 1.428x+2.823
	24	26.66±4.60	42.60±4.60		
SF-3	12	18.60±12.20	26.60±14.00	94.43	y = 0.764x+3.491
	24	25.30±6.10	33.33±6.10		
SF-4	12	24.00±10.50	36.00±12.00	44.73	y = 0.996x+3.356
	24	29.30±8.30	40.00±10.50		

SF1-SF4: Four (4) sub fractions derived from the purification of DEESE-f1 (*Dacryodes edulis* ethanol seed extract fraction 1).
Mortality values represent Mean±SD of 3 replicates of r=25

4.9 Structural Analysis II

4.9.1 Gel filteration

The gel filteration of the *Carica papaya* hexane leaf extract fraction 1 sub fraction 3 yielded two (2) distinct fractions with R_f values 0.66 and 0.75. The fractions were subjected to larvicidal testing on the larvae of *Aedes vittatus* and fraction 2 was selected for NMR analysis.

4.9.2 Larvicidal activity of fractions 1 and 2 of sub fraction 3 of CPHLE-f1

Table 4.33: Larvicidal activity was carried out on fraction 1 and 2 of sub fraction 3 of CPHLE-f1. LC_{50} values at 48 hours for fraction 1 and 2 were 3.90 and 2.25ppm respectively

4.9.3 Nuclear Magentic Resonance of fraction 2

The NMR shows the presence chemical shifts suggestive of CH_3 -10.198ppm, CH_2-18.753-34.724ppm, 64.187- CH group attached to an OH (hydroxyl) group while 124.842-126.958 – C=C (carbon double bond).

Figure 4.3

Suggested structure of the compound in fraction 2. An eighteen carbon fatty alcohol octadec-9-enol $CH_3(CH_2)_{14}CH{=}CHCH_2OH$ with molecular weight of 268 g/mol.

Octadec-9-enol is the proposed parent compound. Fragmentation patterns showed a peak with molecular weight 98g/mol with formula C_7H_{15} equivalent to 99g/mol with loss of one proton.

M/Z = [M+1]

[98+1] = 99

The other suggested half of the fragmentation of the parent compound is one similar to dodecyl acrylate with molecular weight of 168g/mol with formula $C_{11}H_{21}O$ equivalent to 169g/mol with the loss of one proton.

M/Z = [M+1]

[168+1] = 169

Table 4.33: Larvicidal activity of fractions 1 and 2 of *Carica papaya* hexane leaf extract fraction 1 sub fraction 3 (CPHLE-f1 sub fraction 3)

CPHLE-f1 Sub fraction 3	**Conc (ppm)**	**Percentage Mortality (%)**		**Lethal Conc (ppm)**
Fractions	**Time (hrs)**	**12.5**	**25**	**LC_{50}**
SSF-1	12	48.00±4.00	80.00±10.50	12.88
	24	80.00±6.10	88.00±0.00	3.90
SSF-2	12	64.00±8.00	100.00±0.00	7.43
	24	80.00±8.00	100.00±0.00	2.25

SSF1-SSF2: Two (2) sub fractions derived from the purification of CPHLE-f1 (*Carica papaya* hexane leaf extract fraction 1) sub fraction 3..
Mortality values represent Mean±SD of 3 replicates of r=25

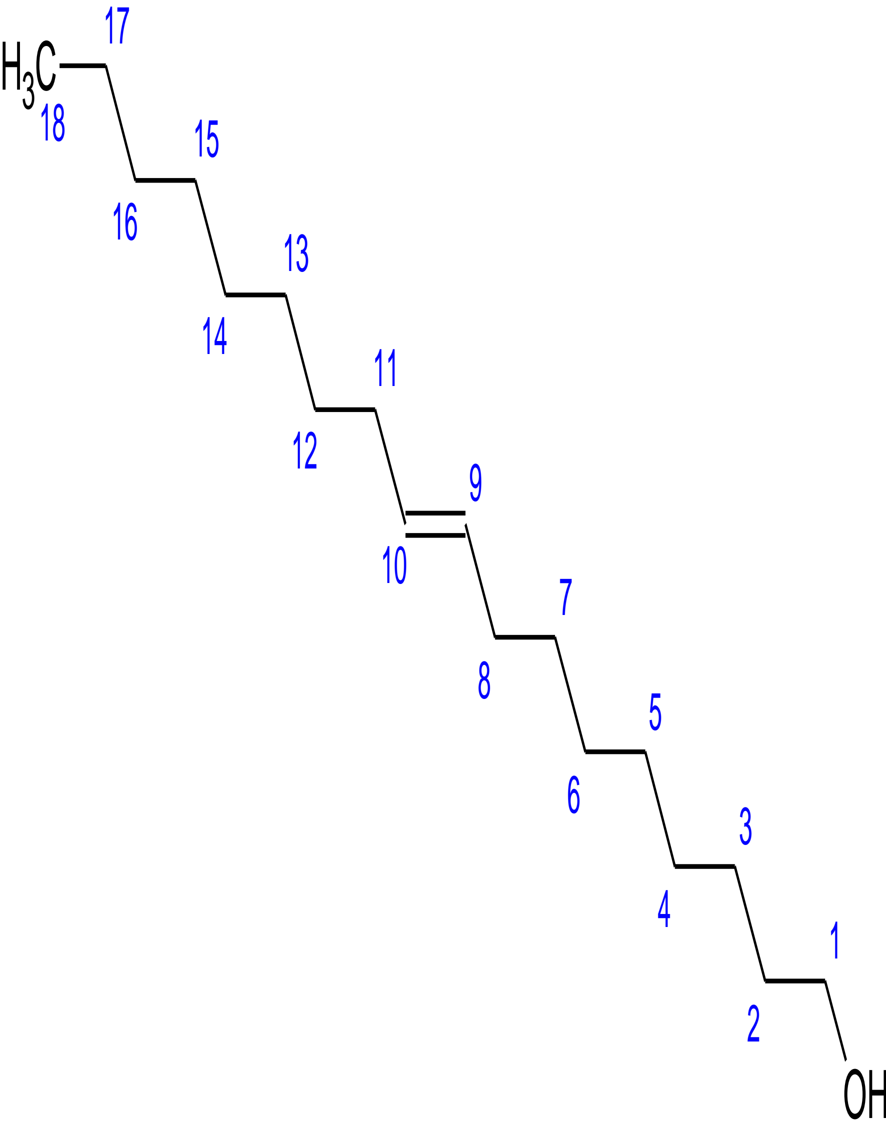

Figure 4.3: Suggested structure of an active principle within *Carica papaya* hexane leaf extract

CHAPTER FIVE

5.0 DISCUSSION

Mosquitoes are ubiquitous and a nuisance responsible for the most vector transmitted diseases known to man such as malaria, filariasis, Japanese encephalitis and most recently the zika virus. They possess economic impact including loss in commercial and labour outputs especially in countries with tropical and sub-tropical climates (Govindarajan *et al.,* 2013). Though mosquitoes can be controlled by using repellants, vector control is currently facing serious challenges due to the emergence of resistance to conventional insecticides (Hayatie *et al*., 2015). Mosquitoes develop genetic resistance to synthetic insecticides and even biopesticides such as *Bacillus sphaericus*. The increased use of these insecticides may enter the food chain and therefore the liver and kidney may be irreversibly damaged. They may even cause mutation of genes that are observable only after several generations. The use of mosquito coils containing synthetic pyrethroids and other organophorous compound cause some side effects such as breathing problems, eye irritation, headache and asthma (Govindarajan *et al.,* 2013). The current study was carried out to determine the effects of the plants *Carica papaya* and *Dacryodes edulis* on the larvae of three mosquito species *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus*. Larvae were chosen over adult mosquitoes because the attack against adults in temporary, unsatisfactory and pollutes the environment (Kamaraj *et al*., 2010). Also larvae are confined to a specific place hence they can be treated in a way and manner that allows for maximum effect as larvicides are known to be able to control mosquitoes in their breeding sites (Kamaraj *et al*., 2010). Extraction was carried out using three solvents, distilled water, ethanol and n-hexane separately. Percentage yield of the twenty-four (24) extracts were determined and

compared. The seed extract of *Carica papaya* gave the highest yield in n-hexane solvent than the aqueous and ethanol extracts which may be due to the high concentration of lipids (Margo *et al.*, 1986; Cassia *et al.*, 2011; Nwofia *et al.*, 2012; Li *et al.*, 2015) that are readily extracted in a non-polar medium. The leaf extract of the plant had the highest yield in the hexane extract than the aqueous and ethanol extract; this may be due to the solubility of the expected constituents of leaves such as cyanogenic glycosides, glucosilonates and flavonoids (Vyas *et al.*, 2014) responsible for pigmentation, alkaloids and saponins (Ayoola and Adeyeye, 2010) in polar solvents. The stems and roots of *Carica papaya* produced their highest yield in the ethanol solvent probably because the stem back contains alkaloids, flavonoids, tannins, phenol, steroids, terpenes and cardiac glycosides (Saidu and Nweri, 2013); while the roots are reported to contain saponins, alkaloids, tannins, glycosides and phenols (Doughari *et al.*, 2007; Iwu *et al.,* 2016). *Dacryodes edulis* seed extract had the highest yield when extracted in ethanol when compared with the aqueous extract and the hexane extract. *Dacryodes edulis* seeds are very rich in free fatty acids as well as tannins, saponins, flavonoids, steroids, alkaloids, cardiac glycosides, phenols and carboxylic acids (Nwokonkwo, 2014). The leaf extract with the highest yield was the ethanol extract when compared with the aqueous and the hexane extracts as the leaves are rich in phenolics such as quercitrin, isoquercitrin, isohamnetin and rhamnoside as well as anthocyanins such as cyanidin and petunidin (Missang *et al.*, 2003) which are readily extracted in alcohol. The stem had the highest yield in the aqueous medium containing phytochemicals such as saponins, tannins and alkaloids (Ajibesin, 2011). The root of *Dacryodes edulis* is reported to contain alkaloids, flavonoids, saponins and tannins (Idu *et al.,* 2014). Previous studies have shown varying degrees of larvicidal activity of different

parts of the plants; however it has been shown in literature that the active components of plants vary with geographical region, age of the plant and plant variety (Sesanti *et al.,* 2014). Extractability of a particular component appears to be dependent on the extraction medium, polarity and the ratio of solute to solvent. Different parts of the same plant may synthesize and accumulate different amounts of a particular compound due to their differential gene expression which affects biological properties of the plant extract (Illoki-Assanga *et al*., 2015).

The seeds of *Carica papaya* at 500 ppm showed activity in all three solvent extracts against the larvae of *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus.* The aqueous extract gave low mortality contrary to Malanthi and Vasugi (2015) where aqueous extract of the seeds of *Carica papaya* gave mortality of 83% against the 2nd Instar and 86% against the 4th instar of the larvae of *Aedes aegypti* at a concentration of 1000 ppm; also at concentrations of 250 mg/l and 500 mg/l of the crude aqueous extract of the seed was reported to have resulted in 100% mortality against *Aedes aegypti* (Hayatie *et al*., 2015). This disparity may be due to the differences in genus and specie and the concentration used. The results of Malanthi and Vasugi (2015) is however reflected in this study with the 100% mortality at 24 hours observed when *Culex quinquefaciatus* was treated with the ethanol extract of the seeds of *Carica papaya* even at a lower concentration. Sesanti *et al*. (2014) reported mortality of 100% against the larvae of *Anopheles gambiae* at 80 ppm when treated with the ethanol extract of the seeds at 48 hours, this was also not reflected here when the specie was treated with a higher concentration of 500 ppm, maximum mortality observed was 76%. Rawani *et al.* (2012) showed that at a concentration of 100 ppm of the ethanol extract of the seeds of *Carica papaya* resulted in mortality of 50% at 48

hours. This disparity from this study which gave 100% against *Culex quinquefasciatus* in the same time frame is likely due to the difference in concentration used and the extraction method since Rawani *et al.* (2012) used successive extraction to the study's use of single extraction. The seeds of *Carica papaya* contain 79.1% fatty acids (Margo *et al*., 1986) and 29.16% lipids and larvicidal activity may reside within these compounds. Nunes *et al.* (2013) are of the opinion that the component of the seed toxic to *Aedes aegypti* is their study is generated by an enzyme tegupain. Interestingly the same study by Nunes *et al.* (2013) found that when the seed of *Carica papaya* was separated into cotyledon and tegument and separately tested, larvicidal activity was lost. The ethanol extract of the leaves of *Carica papaya* at concentrations of 5% (w/v) and 10% (w/v) gave mortality of 20% and 35% after 24 hrs (Oladimeji *et al*., 2007). The aqueous extract of the leaf of the plant gave 100% mortality against the larvae of *Anopheles gambiae* and *Culex quinquefasciatus* at 0.06 mg/ml and 0.10 mg/ml (Okolie, 2006). This result was different from what was gotten in this study probably due to the age of the plant or differences in method of extraction. The study by Malathi and Vasugi (2015) of the aqueous extract of the leaf showed 26% and 20% mortality against 2nd and 4th instar *Aedes aegypti* respectively, results also different from those reported by Okolie (2006) but specie differences here may be the principal factor responsible for the disparity. The results by Sesanti *et al.* (2014) on the effect of the ethanol extract of the leaf on *Carica papaya* on *Anopheles gambiae* however was consistent with that of the study where 97% mortality was observed in the study and 100% observed by Sesanti *et al*. (2014) at the same concentration of 500 ppm. The crude ethanol extract of the leaf of *Carica papaya* at 500 ppm against *Culex quinquefasciatus* gave mortality of 43% at 24 hours (Ravichandran *et*

al., 2014). Modified seed and leaf granules of *Carica papaya* tested on *Aedes aegypti* produced 95% mortality at 48 hours (Wahyumi, 2015). The hexane and ethanol extracts of the seed and leaves of *Carica papaya* displayed better larvicidal activity than the aqueous extract which is similar to the results derived from the investigations of *Cassia occidentale* and *Lantana camara* (Seroro and Anofi, 2016). The stems showed highest mortality in aqueous medium when compared to ethanol and hexane with insignificant difference between the three larval species. The results are similar to the results observed in the study of Oladimeji *et al.* (2007) where the ethanolic extract of the leaf, stem and root were tested on *Aedes aegypti* and mortality observed in the stem treated group at 24 hours at 5% (w/v) was 30%. The stems of other plants in different solvent media have proven effective against different mosquito species; some of which are the hexane extracts of the stems of *Achyranthes aspera, Cassia occidentalis, Catharanthus roseus*, *Lantana camara* and *Xanthium strumarium* against the early fourth instar of *Aedes aegypti* at a concentration of 1000 ppm at 24 hours with mortality values of 100% in all cases (Sharma *et al.,* 2016). The aqueous extract of leaf, bark, stem and root of *Jatropha curcus* against *Anopheles gambiae* showed 100% mortality at 1000 ppm, however at 500 ppm only the aqueous extract of the bark gave the same result (Ohimain *et al.,* 2014). The effect of the roots of *Carica papaya* against the 4th instar larvae of *Anopheles gambiae* was shown to have mortality of 70 and 80% at 12 and 24 hours respectively at 10% (w/v) (Oladimeji *et al*., 2012). This differs from the results of this study where there was no effect observed in the larvae of all three species when tested with aqueous, ethanol and hexane extract of the roots of the plant. The difference between the results of Oladimeji *et al.* (2012) and this study may be due to the higher concentration used by Oladimeji *et al.* (2012), the extraction time and the difference

in methodology where larvae were transferred into recovery cups before mortality determinations may have also contributed to the mortality observed as opposed to mortality being determined in the test containers as was done in this study.

The ethanol extract of the seeds of *Dacryodes edulis* gave the highest mortality against the three larval species than aqueous and hexane extract though the hexane extract of the seeds gave insignificant difference when compared to the effects of the ethanol extract against *Culex quinquefasciatus.* The seed of *Dacryodes edulis* is rich in fatty acids such as oleic acid, palmitic and linoleic acid (Obasi and Okolie, 1993) which may be responsible for its larvicidal activity and these are extracted in both ethanol and hexane (Grima *et al*., 1994). They also contain secondary metabolites such as alkaloids and tannins which make up the highest concentration in the seeds (Ujowundu *et al*., 2010). Fatty acids have been shown to be effective larvicides (Silva *et al*., 2015) and so have alkaloids (Talontsi *et al.,* 2011; Lui *et al*., 2012) and tannins (Silva *et al*., 2004). The hexane extract of the leaf of *Dacryodes edulis* gave the highest mortality against the three larval species at 500 ppm. However the ethanol extract gave mortality values of 82% at 48 hours against *Culex quinquefasciatus.* The results were similar to the results of Oladimeji (2011) on the larvicidal activity of the leaves of the plants on *Anopheles gambiae* which gave mortality of 90% at 24 hours. But the effect of the stems reported by the same author where 100% mortality was observed at 24 hours at a concentration of 10%w/v was dissimilar from the results gotten from this study where no effects was observed. This may be attributed to the differences in the test concentrations used, area of collection, season of the year and maturity of the plant. The roots of the plant also showed no larvicidal activity. Ethanol and hexane extracts of the leaf and seed of *Carica papaya* and the hexane extract of the leaf and the ethanol extract of the

seed of *Dacryodes edulis* were selected to further evaluation because they conformed with the preset criteria of effective extracts to be considered were those that caused mortality of above 70% against at least two (2) of the larval species.

The potentialities of the selected extracts were evaluated using the LC_{50} values and it was observed that it reduced with time in all test cases. This shows that the effectiveness of the extracts increased with exposure time. The ethanol extract of the leaf of *Carica papaya* gave 93.19, 40.80 and 43.91 ppm against the larvae of *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* at 48 hours. The results against *Anopheles gambiae* in this study showed better potentiality than results reported by Sesanti *et al.* (2014) probably because the data was collected at maximal time of 24 hours as opposed to 48 hours used in this study and differences in solvent concentrations may also be a factor. The methanol extract of the leaf tested on *Aedes aegypti* by Kalimunthu *et al.* (2012) showed an LC_{50} value of 375.8 ppm which was much higher than those observed in this study, the differences however could be based on the usage of methanol, differences in species and plant extraction. The hexane extract of the leaf of *C.papaya* gave LC_{50} values of 48.14, 29.32 and 74.83 ppm at 48 hours against *Aedes vittatus, Anopheles gambiae* and *Culex quinquefasciatus* larvae respectively. The LC_{50} values of the hexane extract of the leaf was lower than that of the ethanol extract of the leaf except when considering their effects on *Culex quinquefasciatus* at 48 hours and there are few studies involving the evaluation of the hexane extract of this plant part. The high potential of non-polar extracts has been demonstrated against mosquito larvae (Krishnappa *et al.,* 2012). Evaluation of hexane extract of *Citrus sinensis* against *C. quinquefasciatus* showed moderate larvicidal activity with LC_{50} of 446.84 ppm (Warikoo *et al.*, 2012), a fraction (fraction D) of the hexane

extract of the leaf of *Murraya koenigi* gave 100, 97.6 and 99.2% mortality at 100 ppm against *Aedes aegypti, Anopheles gambiae* and *Culex quinquefasciatus* with LC_{50} values of 35.06, 27.20 and 42.51 ppm at 24 hours (Arivoli *et al*., 2015) and Nzelibe and Chintem (2015) reported the hexane extract of *Ocimum gratissimum* leaf gave LC_{50} values of 2.5 ppm at 48 hours. The hexane extract of the leaf of *Abutilon indicum* tested on *Aedes aegypti* showed 100% mortality at 1000ppm with LC_{50} value of 261.31 ppm (Arivoli and Tennyson, 2011), further giving credence to the effectiveness of hexane extracts of the leaf of different plants against the larvae of different species. The ethanol extract of the seed of *Carica papaya* gave similar results with those reported by Sesanti *et al*. (2014) against *Aedes vittatus* and *Anopheles gambiae.* The ethanol extract of the seeds also gave similar results to those reported by Rawani *et al.* (2012). The hexane extract of the seeds gave good to moderate LC_{50} values of between 61.47-264.78 ppm at 48 hours. The ethanol and hexane extract of the seed of *Persea americana* against *Aedes aegypti* gave a similar pattern to the ethanol and hexane extract of the seed of *Carica papaya* against *Aedes vittatus* with the hexane extract having a lower LC_{50} value of 9.82 mg/l than the ethanol extract with LC_{50} value of 21.32 mg/l (Torres *et al.,* 2014). Hexane extract of the seed of *Persea americana* gave LC_{50} values of 2.37 and 8.87 mg/ml against the fourth instar of *Artemia salina* and *Aedes aegypti* (Leite *et al*., 2009). The hexane extract of the seed of *Persea americana* against *Aedes vittatus* also showed the efficiency of non polar solvent in extracting larvicidally active components with 100% mortality at 50 ppm six hours post exposure with LC_{50} value of 0.829 ppm (Nzelibe and Albaba, 2015). Generally the results of larvicidally active extracts of *Carica papaya* showed that the smaller the concentration of the extract(s) used the longer the contact time required with the extract(s) and vice versa.

The difference in results with other studies may be based on maturity of the leaves, papaya varieties, method of extraction and concentrations used (Sesanti *et al*., 2014). *Dacryodes edulis* leaf hexane extract gave LC_{50} values of 1177, 1762.1 and 508.28 ppm at 48 hours against *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* respectively. These results show that a higher concentration of the extract is required to bring about the required larvicidal action. The ethanol extract of the seed on the other hand gave LC_{50} values of 150.54, 85.99 and 110.18 ppm at 48 hours, against *Aedes vittatus, Anopheles gambiae* and *Culex quinquefasciatus* showing higher potentiality. The activity of the seed may be due to the lipids and fatty acids they contain such as unsaturated fatty acids oleic acid, linoleic acid and linolenic acid (Ajibesin, 2011) are reported to be highly toxic to mosquito larvae. The family Burseraceae to which *Dacryodes edulis* belongs which consists approximately of 700 species and 19 genera has members that have shown larvicidal activity such as *Commiphora molmol* which has been reported to be active against *Culex pipiens* (Murty *et al.,* 2016). Acetylcholinesterase is the main site of action of flavonoids, oleic and palmitic acid. Linoleic acid and linolenic acid might act on both acetylcholinesterase and octopaminergic receptor (Haribalan *et al*., 2015). The processes by which mortality is caused in larvae include activity interfering with the molting process which are under the influence of the ventral nerve cord neurosecretory cells, which release a tanning hormone which may act on these cells or directly on epidermal cells responsible for the production of the enzymes for the tanning or cuticular oxidation process (Vinayagam *et al.,* 2008). Others include compounds that mimic the shape and structure of hormones vital to the lives of insects. The bodies of these insects absorb the compounds and they block the endocrine systems. These result in behavioural and physiological

aberrations that leave the insects confused and unable to reproduce resulting in the plummet of their populations. Insect growth regulatory activity where compounds weaken the cuticle defense system of the larvae causing easy penetration of pathogenic organisms into the insect system (Dua *et al.,* 2009).

Thin layer chromatography of *Carica papaya* hexane leaf extract which was selected alongside *Dacryodes edulis* ethanol seed extract using TLC gel plates F_{254} yielded twelve (12) spots with hexane: ethaylacetate: methanol (8:2:1) solvent system while DEESE yielded seven (7) spots with chloroform: ethylacetate solvent system (7:3).

Column chromatography of *Carica papaya* hexane leaf extract (CPHLE) yielded fractions that were evaluated for larvicidal activity against *Aedes vittatus* and *Culex quinquefasciatus.* The twelve fractions derived produced larvicidal activity of varying potential ranging from 10.72ppm and 335.97ppm against *Aedes vittatus* and 15.15ppm and 196.03ppm against *Culex quinquefasciatus*. These results suggest that the fractions may contain different constituents that act both synergistically and alone as the secondary compounds of plants make up a vast repository with wide range of biological activity (Deore and Khadabadi, 2009). The LC_{50} values gotten from the seven (7) fraction of DEESE gave between 10.48ppm and 106ppm against *Aedes vittatus* and 10.16ppm and 317.58ppm against *Culex quinquefasciatus*. The larvicidal activity of the fractions varied indicating the presence of different compounds with larvicidal activity within the extract. Fractions 1 of both extracts had the highest larvicidal activity reflected by the lowest LC_{50} values against both larval species and were selected for further studies. Fraction 1 for both extracts were derived with 100% of a non polar solvent (hexane and chloroform) and the larvicidal effect of a non polar fraction of an alcohol extract of the seed of a plant was

exhibited in the effect of the fractions of *Buccholzia coriacea* seeds on *Anopheles gambaie* where 100% mortality was observed in the chloroform fraction of the methanol extract against the third and fourth instar of *Anopheles gambiae* at concentrations of 1000, 500 and 250 ppm (Fred-Jaiyesinmi *et al*., 2011b).

Phytochemical analysis of CPHLE-f1 yielded the presence of carbohydrate, cardiac glycosides and steroids with the exception of saponins, tannins (Kavinandam, 2016) and alkaloids (Ikeyi *et al.,* 2013) reported to be present in the crude of the leaf of the plant. The absence of alkaloids and tannins in the hexane fraction tallys with the analysis carried out by Juarez-Rojop *et al.* (2014). Analysis of DEESE-f1 showed presence of carbohydrate, cardiac glycosides, steroids, tannins and alkaloids with the absence of phytochemicals like saponins and flavonoids (Nwonkonkwo, 2014) reported to be present in the seeds. The analysis carried out by Anyam *et al.* (2015) showed the ethanol extract of the seeds tested contained saponins and steroids with the absence of alkaloids, tannins, flavonoids, carbohydrate and cardiac glycosides. Alkaloids, saponins, steroids, terpenoids, phenolics, tannins and essential oils are known to possess pesticidal properties (Gutierrez *et al.,* 2014; Torres *et al.,* 2015). The flavonoids in *Carica papaya* are reported to lower the appetite in larvae (Sesanti *et al*., 2014). Isolated flavonoids from aqueous extracts of *Annona squamosa* was found to be effective up to 80% mortality against *Callosobruchus chinensis* (Kotkar *et al.,* 2002). Saponins on the other hand act by causing haemolysis of the blood vessels of the larvae, lowering food intake, retardation in development, instability in development and declining reproduction (Gubitz *et al.,* 1999). Biologically active terpenoids of *Tagetes minuta* such as dihydrotagetone, tagetones, ocimenones and piperitone have been reported to have possible synergistic larvicidial effects (Seroro and

Anofi, 2016). Organic fatty acids in *Carica papaya* seed extract has been reported to inhibit the process of metamorphosis (Sesanti *et al.,* 2014).

The deleterious effects of plant extracts or pure compounds on insects can be manifested in several manners including toxicity, mortality, antifeedant, growth inhibitory, suppression of reproductive behavior and reduction of fecundity and fertility (Chinnamani *et al.,* 2016). Mortality and LC_{50} values of the selected fractions of the two extracts against the two mosquito species were tested on 1st to 4th instar of the larvae. It was observed that mortality increased with advancement in development i.e. mortality against 3rd and 4th instars were higher than 2nd and 1st instars except DEESE-f1 on *Culex quinquefasciatus.* This result is contrary to those reported by Kovedan *et al.* (2012a) where mortality reduced with larval development. The differences may be due to concentration, solvent and larval species. The adult emergence result of the study was used to determine growth inhibitory activity of CPHLE-f1 and DEESE-f1 which were tested on 1st to 4th instars of *Aedes vittatus* and *Culex quinquefasciatus*. Activity of CPLHE-f1 on *Aedes vittatus* showed increased inhibition with instar with the 1st, 2nd, 3rd and 4th instars showing 14.84, 43.14, 48 and 60% inhibition respectively showing the highest growth inhibition activity seen in the late third-early fourth instar. This was also observed when CPLHE-f1 was tested on *Culex quinquefasciatus.* The effects of DEESE-f1 on *Aedes vittatus* was such than no inhibition occurred in the 1st instar with insignificant inhibition on the 2nd instar with inhibition of the 3rd and late 3rd-early 4th being similar with their inhibition values being 0, 0.76, 44.43 and 46.67% against 1st, 2nd, early 3rd and late 3rd - early 4th instars respectively. In the growth inhibitory assay of DEESE-f1 on *Culex quinquefasciatus* there was no inhibition on the 2nd instar with minimal inhibition of the 1st instar and the 3rd and 4th instar showing moderate

to high inhibition with values of 4.31, 34,18 and 65.78% against 1st, early 3rd and late 3rd-early 4th instar respectively. There was clear prolongment of the larval stages in the treated groups and this is similar to the inhibition of adult emergence of *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* by the leaf extracts of *Cleistanthus collinus* (Arivoli *et al.,* 2015). The results of the the growth inhibition of *Aedes aegypti* by oleic acid has been reported at a concentration of 100µl/ml (Perez-Gutierrez *et al*., 2011). Rajumar and Jebanesan (2005) reported the growth inhibitory activity of *Centella asiatica* on 3rd instar larvae of *Culex quinquefasciatus* and it showed inhibition activity of 91.2 - 98.8% between 19-31 degrees. Insect growth regulation properties of plant extracts are very interesting and unique in nature since insect growth regulator works on the growth hormone. The enzyme ecdysone plays a majoe role int the moulting of larval stages of an insect. When the active plant compounds enter into the body of the larvae, the activity of ecdysone is suppressed and the larvae fails to moult, remaining in the larval stage and ultimately dying (Chinnamani *et al.,* 2016). Adult emergence study carried out on *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* larvae revealed increased larval duration across all treated individuals with decrease in adult longevity (Arivoli and Tennyson, 2011). The growth inhibition activity may be due to the presence of the phytocompounds identified including alkaloids, tannins and flavonoids existing in the plants, which may jointly or independently contribute to produce adult emergence inhibition activity (Arivoli and Tennyson, 2011). Against *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* the ethylacetate fractions of *Calophyllum inophyllum* seed and leaf, *Solanum suratense* and *Samadera indica* leaf extracts and the petroleum ether fraction of *Rhinocanthus nasutus*

leaf extract decreased fecundity over the control ranging from 62.4 and 87.4% at EC_{50} (Muthukrishnan and Pushpalatha, 2001).

Many plants have been reported as interesting sources of AChEI (Feitosa *et al.,* 2011). Presence of acetylcholineseterase activity was evaluated and it was observed that CPHLE-f1 at concentrations of 6.25-100ppm showed a concentration dependent inhibition with 100ppm having the highest inhibition of 31.02 and 31.26% against *Aedes vittatus* and *Culex quinquefasciatus.* The results obtained are not surprising in that where acetylcholinesterase inhibition exists, the inhibition increases with higher concentration as in the study by Da Silva *et al.* (2014) where acetylcholinesterase activity decreases with increase in test concentration though the differences against the the four stages of *Aedes aegypti* was insignificant. The 70% ethanolic extract of the seeds of *Carica papaya* evaluated for acetylcholinesterase activity at 500µg/ml resulted in inhibition of 41.16% (Cyril-Olutayo *et al*., 2011) showing the ACHE potential of the plant but the differences observed from the study may be due to concentration and part of plant used. The concentration dependent inhibition in activity was also reflected in *Melaleuca alternifolia* essential oil against L3 larvae of *Anisakis simplex* (Gomez-Rincon *et al*., 2014). This suggests that the extract prevents binding of ezyme and substrate either by competition for the active site or by conformational change that increases with increase in extract concentration. However, the same analysis carried out on DEESE-f1 yielded a different result where maximal inhibition was observed at 6.25 ppm with a value of 10.68% and 25ppm with inhibition of 22.4% against *Aedes vittatus* and *Culex quinquefasciatus* respectively. Against *Aedes vittatus* the fraction may function by binding at one site of the enzyme and causing a conformational change that presents the other site for substrate

binding thereby reducing the inhibition of the enzyme. In the case of *Culex quinquefasciatus* the fraction may have acted in the same way as for *Aedes vittatus* but at a higher concentration of 25ppm. The crude methanolic and ethanolic extracts of *Jatropha gossypiifolia* L. and *Senna alata* (L.) Roxburgh showed IC_{50} values of 0.05 and 0.08 mg/ml in a microplate acetylcholinesterase inhibition assay (Feitosa *et al.,* 2011) reaffirming the potential of plants as acetylcholinesterase inhibition agents. Few reports communicate the AChE inhibition activity of essential oils which are rich in monoterpenoids (hydrocarbons, alcohols and ketones) and the presence of a of a double bond in structure of a hydrocarbon seem to show strong inhibition in AChE activity (Jyotshna *et al.,* 2015). Structural features are important for biological activity. The presence of a conjugated double bond and an hydroxyl group seems to be important in the AChE inhibitory activity of *E*-anethole and eugenol essential oils from *Croton zehntneri* (Santos *et al.,* 2010).

Mosquito predators such as *Gambusia affinis, Poecelia reticulata* and *Diplonychus indicus* are non target organisms of larvicides (Sivagnaname and Kalyanasundaram, 2004). The effects of concentrations at which 50% mortality of the larvae was observed, LC_{50} and LC_{90} were tested on *Poecelia reticulata* a non target organism of similar habitat and the results were insignificantly different ($p>0.05$) from negative controls, these results are expected as one of the arguments in favour of usage of plants and plant metabolites as larvicides are their non toxic effects on non target organisms (Singh *et al*., 2011; Ray *et al*., 2014; Rawani *et al*., 2014). It has also been reported that fish farmers mash papaya leaves and place them in propagated fish ponds to prevent microbial growth and fungi that are capable of interfering with growth in channel catfish (Sesanti *et al.,* 2014). This lays credence to the specificity of plant metabolites as larvicides. The study by Sivagnaname

and Kalyanasundaram (2004), on the effects of methanolic extract of *Atlantia monophylla* on target organisms, showed that that predatory fish of the same habitat *Gambusia affinisi* and *Poecelia reticulata.* Similarly the crude extracts of *Alternanthera sessilis, Trema orientalis, Gardenia carinata* and *Ruellia tuberosa* showed no mortality when tested on *Diplonychus annulatum* and *Chironomus circumdatus,* non target organism of the same habitat (Rawani *et al.,* 2014).

Fourier transform infra-red spectroscopy of CPLHE-f1 and DEESE-f1 showed presence of functional groups that indicate the likely presence of O-H, C-H, C=O, N-O and C=C groups which correspond with the results of the phytochemical analysis. Carbohydrates are hydroxyl containing compounds that exist with carbonyl C-O, ketone C=O, aldehyde C-H-O and acid COOH. Alkaloids are nitrogen containing plant compound that can exist with oxygen, sulphur, chlorine, bromine and phosphorus (Saxena *et al*., 2013). Flavonoids exist as aglycone, glycosides and methylated deriviatives (Kumar and Panday, 2013). Tannins are polyphenolic compounds containing hydroxyls and carbonyls that can form complexes with various macromolecules are attached to each other via C-C or C-O-C bonds (Juha-Pekka and Maarit, 2011). Steroids and triterpenes are derived from a 30 carbon compound (squalene) 2, 3, epoxide (Sultan and Rauf Raza, 2015). Structural and functional group variations have been identified as possible contributing factors to larvicidal activity as in the study by Kweka *et al.* (2016) showed that oxygenated monoterpenes exhibited stronger larvicidal effects than monoterpene hydrocarbon. The position of the double bond in the *p*-menthane skeleton apperared to influence the larvicidal potency of monoterpenes against *Anopheles gambaie.* Carboxylic acid stretch O-H recorded in extracts of *Ocimum* species is considered as one of the functional groups of the five potential bioactive compounds

responsible for the larvicidal activity of the plant against *Culex quinquefasciatus* (Pratheeba *et al.,* 2015). It has been reported that the presence of an exocyclic carbonyl group and conjugatedthe C-C double bond appears to contribute to larvicidal activity; also α,β-unsaturated or conjugated aromatic compounds contribute to biological activity (Lima *et al.,* 2014).

Gas Chromatography Mass Spectroscopy gave the presence of eleven (11) peaks for CPHLE-f1 and nine (9) peaks for DEESE-f1. The major proposed compounds in CPHLE-f1 and DEESE-f1 dodecanol 28.10%, 1-hexadecanol, 2 methyl 31.64% and cyclohexanone 18.93%, 1-decanol 18.45%, dodecyl acrylate 31.84% respectively. Cyclohexanone with percentages of 18.93% and 5.63% in CPLHE-f1 and DEESE-f1 respectively is indicated in anticancer activity. Members of the group such as cyclohexanone from curcumin towards estrogen receptor negative cancer cells showed increased cytotoxic potency (Somers-Edgar *et al*., 2011), they have also been indicated in antifeedant activity (Dancewicz *et al*., 2011). Tetradecane has been shown to have anti fungal anti bacterial activity (Akpuaka *et al*., 2013). Hexadecanoic acid has been found to posses antioxidant activity (Sethi *et al.,* 2013). Different types of alcohols, their derivatives and some lipophilic or amphiphilic compounds are known to exhibit anti-mycobacterial activity and these studies showed that the antimicrobial activity is influenced by the number of carbon atoms present in the alkanol chain (Mukherjee *et al*., 2013) thus activity may be linked to chain length. Another argument was put forward by Haribalan *et al*. (2015) in favour of linking structure and activity, suggesting a relationship in which the degree of saturation, chain length and geometric isomerism of fatty acids appearing to play a role in fatty acid larvicidal toxicity. Phenol, 2-4-bis (1,1-dimethylethyl) with 8.16% in CPHLE-f1 and 4.97% in DEESE-f1

with molecular formula $C_{14}H_{22}O$ has good antioxidant activity (Yogeswan *et al*., 2012). Other compounds that have been linked with larvicidal activity include phytol, a diterpene reported to have antibacterial activity against *Staphylococcus aureus* is suspected to be one of the compounds responsible for the larvicidal activity of methanol extract of *Lantana camara* Linn against *Aedes aegypti* and *Culex quinquefasciatus* (Kumar and Maneemegalai, 2008). It has also been reported as an active larvicidal compound in *Pongamia pinnata* against *Spodoptera litura* (Tharan *et al.,* 2016). Dibutyl phthalate from the stem of *Ipomoea carnea* giving 100% mortality against *Aedes aegypti* and *Culex quinquefasciatus* at a concentration of 300 ppm at 24 and 48 hours (Khatiwora *et al*., 2014). The compound 1,2- benzenedicarboxylic acid , mono (2-ethyl) ester isolated from the leaf of *Clausena anisata* gave 100% mortality at 40ppm against the larvae of *Aedes aegypti* and *Anopheles stephensi* (Jayaraman *et al.,* 2015). The bioactive compound β-thujaplicin derived from *Chamaecyparis obtuse* leaf extract showed strong larvicidal activity against *Aedes aegypti, Aedes togoi* and *Culex pipiens pallens* (Sutthanont *et al.,* 2010). CPHLE-f1 and DEESE-f1 were further purified and they yielded five (5) and four (4) sub-fractions respectively. The sub fractions were subjected to larvicidal testing against the larvae of *Aedes vittatus* and sub fraction 3 of CPHLE-f1 produced the highest larvicidal activity when compared to the other four (4) sub-fractions. The sub-fractions of DEESE-f1 however gave LC_{50} values that were higher than that of the original fraction 1 (f1). This suggests that the components within the fraction are more effective when acting together than alone i.e synegestic activity. Maurya *et al*. (2012) suggested activity of non lethal concentrations of larvicidal extract combined with synthetic pesticides can increase the overall activity of the combination by a mechanism of synergism which may be due to the

phytochemicals inhibiting the ability of mosquito larvae to employ detoxifying enzymes against synthetic chemicals. The combination of temephos and extract of *Cuscuta reflexa* revealed a synergestic action against both anopheline and culicine larva at ratio 1:1 than when each were tested individually. It is believed the action here is based on the possibility that the plant extract inhibits some factors that can act against synthetic chemicals (Bhan *et al.,* 2015). It is believed that the use of synergists in mosquito control programs is not only more economical but will minimize the induction of resistance in the mosquito population and will apparently continue to render the extracts effective as pest control agents (Yankanchi *et al.,* 2014).

Gel filtration of sub-fraction 3 of CPHLE-f1 yielded two spots, which were tested for larvicidal activity against *Aedes vittatus* with LC_{50} values of 3.9 and 2.55ppm respectively. Sub fraction 2 was selected for NMR analysis.

Nuclear magnetic resonance of fraction 2 of sub-fraction 3 of CPHLE-f1 was determined and the ^{13}C NMR showed ten (10) peaks. Peaks at 126.958 and 124.842 are indicative of a carbon double bond, while the peaks at 18.753-34.724 suggest carbon single bonds, the peak at 10.198 suggest a methyl group while the peak at 64.187 suggests a hydoxy group. Each peak identifies a carbon in a different environment within the molecule. Carbons that exist in the same environment will present as a single peak and the double bond will exert its effect up to four (4) bonds to the left and to the right of it. Suggestion of structure is a fatty acohol Octadec-9-enol. Fatty alcohols have been indicated in larvicidal activity such as fatty alcohols extracted from *Zingiber officinale* (ginger) showed anti-larval activity against *Anisakis simplex* (Lin *et al*., 2010), coconut fatty alcohol showed larvicidal activity against *Aedes aegypti* with LD_{50} value of 200ppm (Mesolania *et al*., 2009). Fatty alcohol 1-

decanol and 1-tridecanol showed antibiting deterent activity similar to N,N diethyl-3-methyl benzamide at 25nmol/cm^2 against *Aedes aegypti.* In the same study 1-tridecanol gave LC_{50} values of 2.1 ppm while 1-dodecanol gave 5.2 ppm against day old larva (Tabanca *et al.,* 2014). The proposed structure octadec-9-enol with formula $CH_3(CH_2)_7CH{=}CH(CH_2)_8OH$ and molecular weight 268g/mol deduced fron the NMR is considered the parent compound and upon fragmentation in the GCMS it is suggested that a single proton each was lost on the two compounds being considered as daughter compounds to the parent compound. Considering the profile of compounds from the GCMS spectrum and their corresponding molecular weights, a compound similar to cyclohexane with molecular weight 98g/mol and formula C_7H_{15} and dodecyl acrylate with molecular weight 168g/mol with formula $C_{11}H_{21}$ are considered possible fragments of the parent compound.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

1. The seed of *Carica papaya* contains phytochemicals that are effective as larvicides against *Aedes vittatus*, *Anopheles gambiae* and *Culex quinquefasciatus* in aqueous, ethanol and hexane solvents but ethanol and hexane extracts gave better larvicidal activity. The trend of effectiveness continued in the leaf extracts but with ethanol and hexane still showing better potential. The stem of *Carica papaya* is moderately effective against the three species while the root showed no activity at the test concentration of 500ppm. The ethanol extract of the seed and leaf of *Dacryodes edulis* were the most potent extract with larvicidal activity against the three larval species.
2. The LC_{50} values of extracts suggest good potentiality for the ethanol and hexane extract of the leaves and seeds of *Carica papaya* and the ethanol extract of the seed of *Dacryodes edulis.*
3. Partial purification of hexane leaf extract of *Carica papaya* yielded twelve (12) fractions with eight (8) fractions giving values below 100ppm and six (6) of the seven (7) fractions of *Dacryodes edulis* ethanol seed extract giving similar results. Fraction one (1) of both extracts gave the best larvicidal activity.
4. Phytochemical analysis using traditional methods showed the presence of carbohydrates, cardiac glycosides, steroids and flavonoids in *Carica papaya* and carbohydrates, cardiac glycosides, steroids, tannins and alkaloids in *Dacryodes edulis.*

5. Growth inhibitory activity was most pronounced in the third (3rd) and fourth (4th) instars of *Aedes vittatus* and *Culex quinquefasciatus* by both CPHLE-f1 and DEESE-f1. Acetylcholinesterase inhibition assay also shows a degree of inhibition aagainst both larval species by both extract fractions.
6. Effect of the fractions on *Poecelia reticulata* showed no effects, suggesting the safety of the fractions to organisms that share habitat with the mosquito larvae.
7. Fourier transform infra red analysis of the fractions showed the presence of similar functional groups between them such as alkane, alkene, nitro compound, oxygen in the form of carbonyl and aldehyde with differences such as CPHLE-f1 containing hydroxy group and DEESE-f1 containing phenyl/alkoxy groups. NMR identified the more active sub-fraction of CPHLE-f1 to be an eighteen carbon compound suggestive of Octadec-9-enol.

6.2 Conclusion

This study confirms the larvicidal activity of *Carica papaya* and *Dacryodes edulis.* It showed that the hexane extract of the leaf of *C. papaya* has as much potential as a larvicide as the ethanol extract of the seed and leaf that are widely reported. *Dacryodes edulis* seed with its rich lipid content can be harnessed for commercial larvicidal use. The mode of action may be but is not restricted to growth inhibitory activity and acetylcholinesterase inhibitory activity and they are non toxic in the aquatic environment due to their inactivity on non target organisms and fatty alcohols are potential larvicides.

6.3 Recommendations

- The effects of selected fractions may be tested on other larval species.
- Pupicidal and adulticidal effect of the fractions may be further evaluated.
- Other larvicidally active fractions and sub-fractions of CPHLE and DEESE may be further analysed to isolate their active princples.
- Combination of active principles may be evaluated to see if activity is increased.

CONTRIBUTION TO KNOWLEDGE

1. This study has established that the ethanol and hexane extracts of the seed and leaf of *Carica papaya* and *Dacryodes edulis* are more effective against the mosquito larval species used than the stem and roots.
2. The lethal concentrations determined showed higher potentiality of CPHLE and DEESE.
3. The most effective larvicidal fractions determined by LC_{50} values were in fraction F1 for both. However the values derived from other fractions of the extracts suggest that other larvicidal active components may also be present in them.
4. The CPHLE-f1 contains cardiac glycosides and steroids while DEESE-f1 contains cardiac glycosides, steroids and tannins.
5. Both fractions showed no toxicity against *Poecilia reticulata* a non target organism of the same habitat suggesting their safety to non-target organisms.
6. Mode of action may be but not restricted to growth inhibitory and acetylcholinesterase inhibitory activity.
7. Fatty alcohols are potential larvicides.

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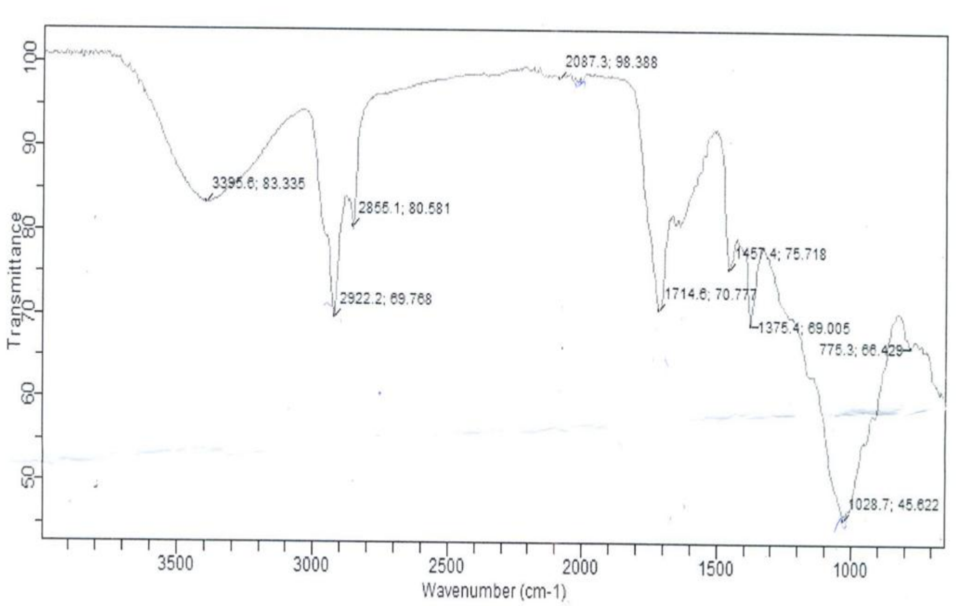
APPENDICES

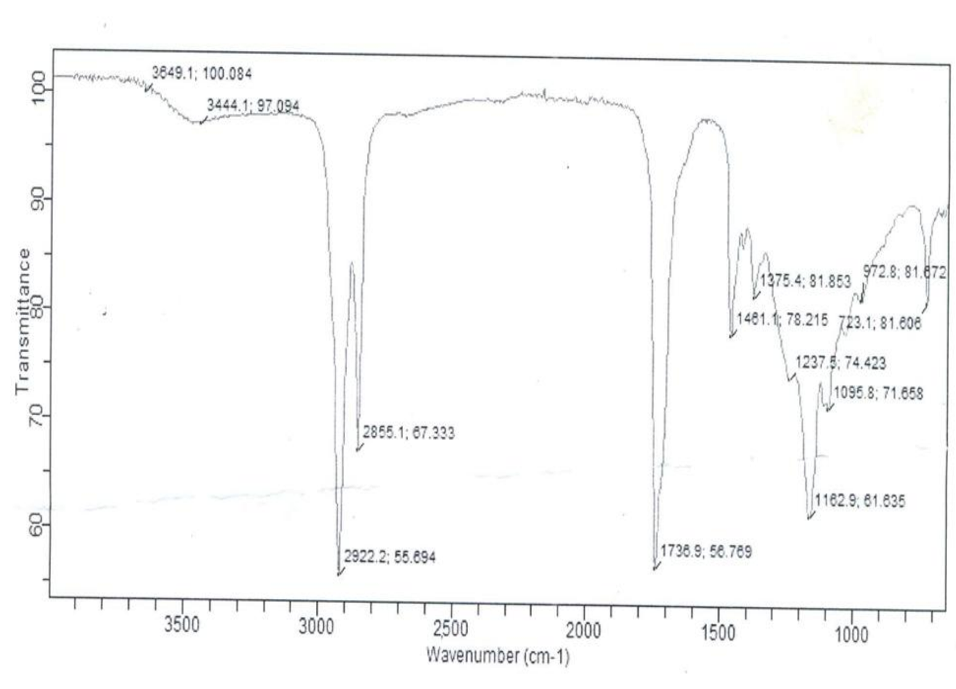
Appendix I

Fourier Transform Infrared Spectroscopy Spectrum of bioactive fraction f1 of *Carica papaya* hexane leaf extract fraction 1 (CPHLE-f1).



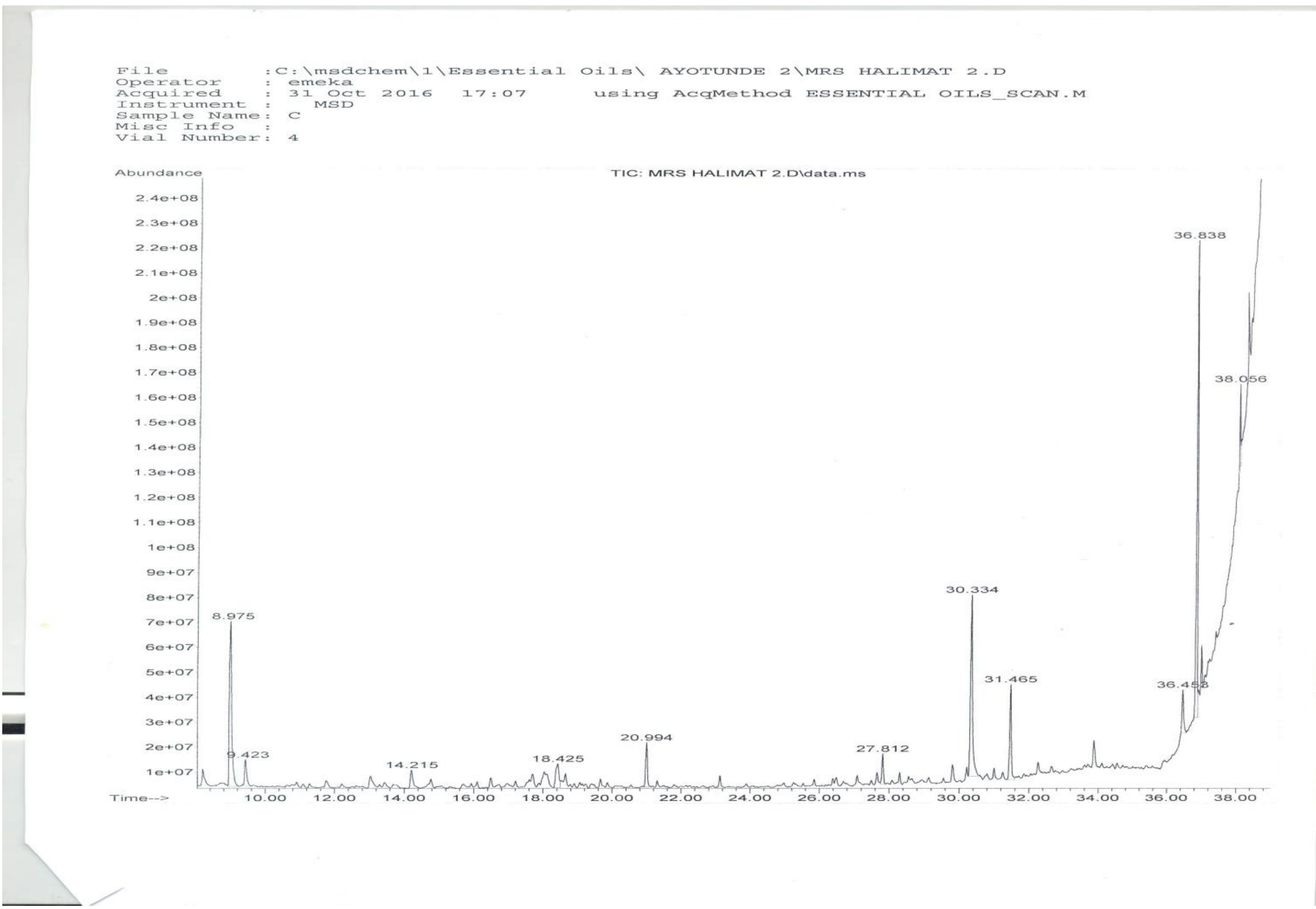
Appendix II

Fourier Transform Infrared Spectroscopy Spectrum of bioactive fraction f1 of *Dacyodes edulis* ethanol seed extract fraction 1 (DEESE-f1).



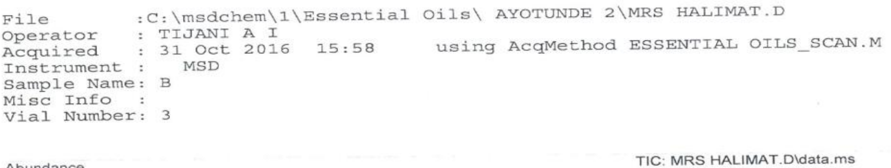
APPENDIX III

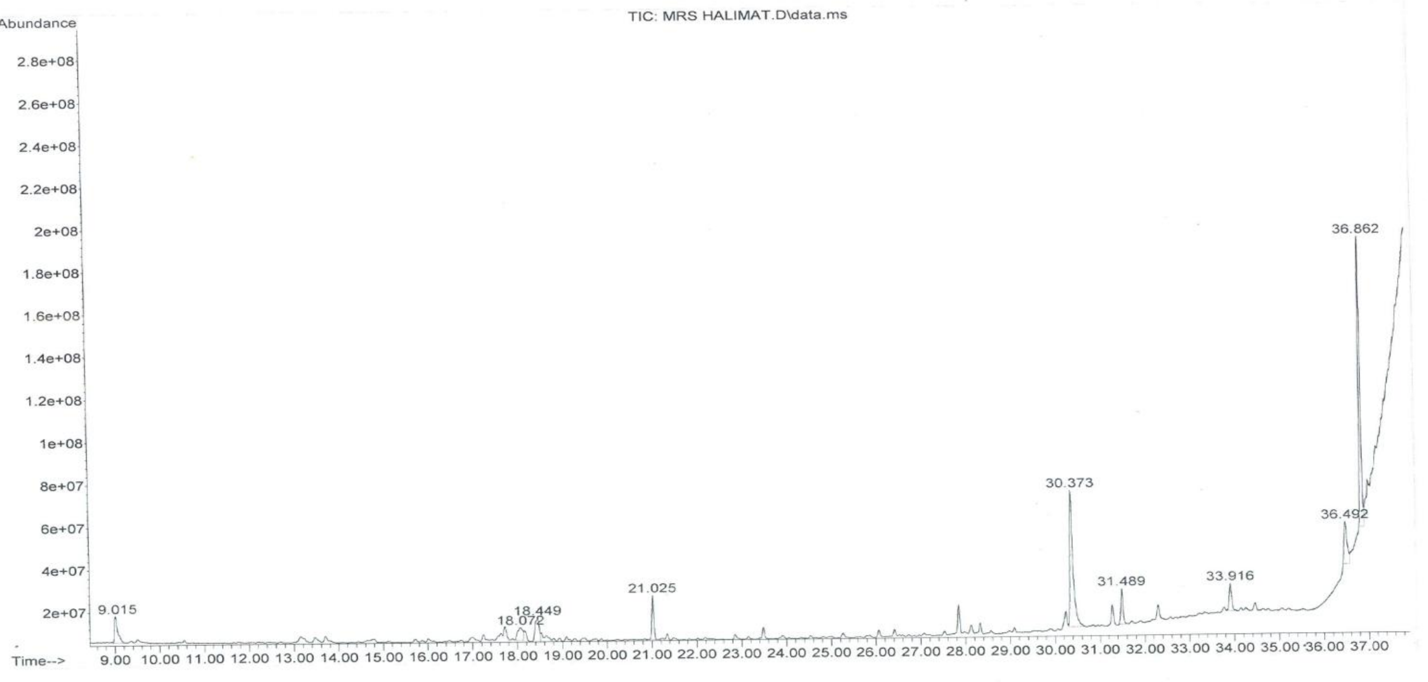
GC-MS of *Carica papaya* hexane leaf extract fraction1 (CPHLE-f1)



APPENDIX IV

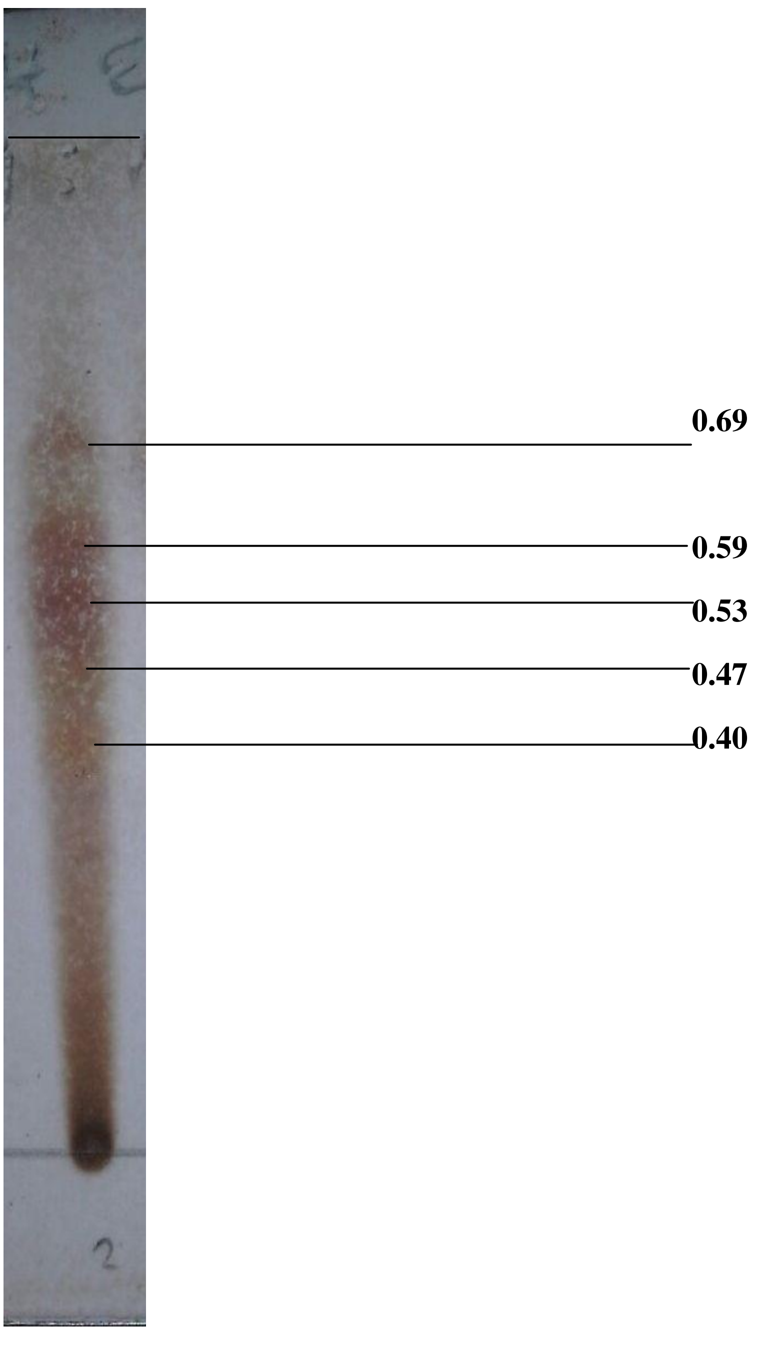
GC-MS of Dacryodes edulis ethanol seed extract fraction1 (DEESE-f1)





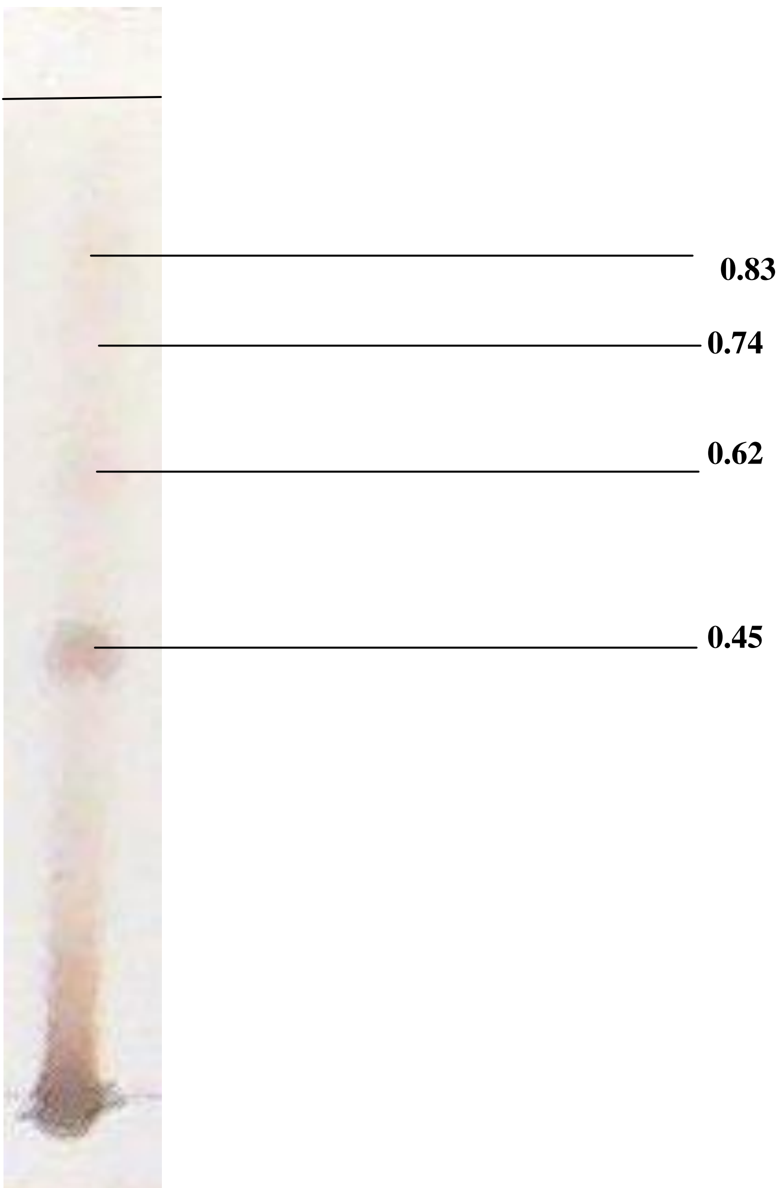
Appendix V

Thin layer chromatography of CPHLE-f1 using hexane:ethylacetate 9:1



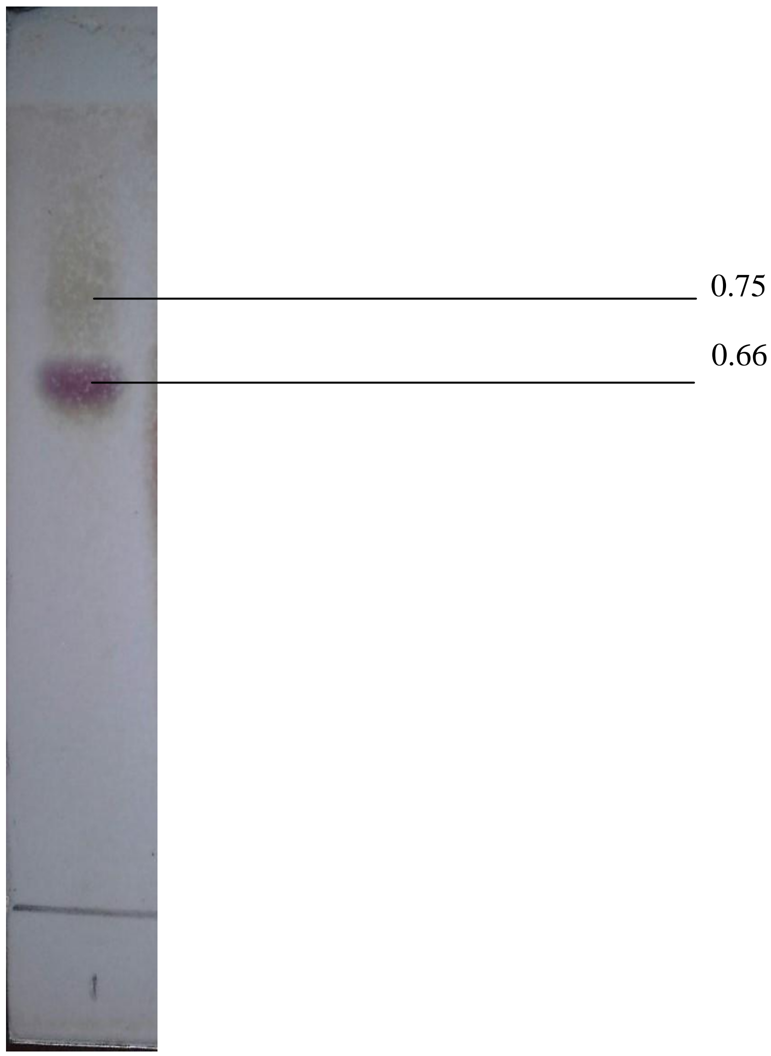
Appendix VI

Thin layer chromatography of DEESE-f1 using chloroform:ethylacetate 9:1



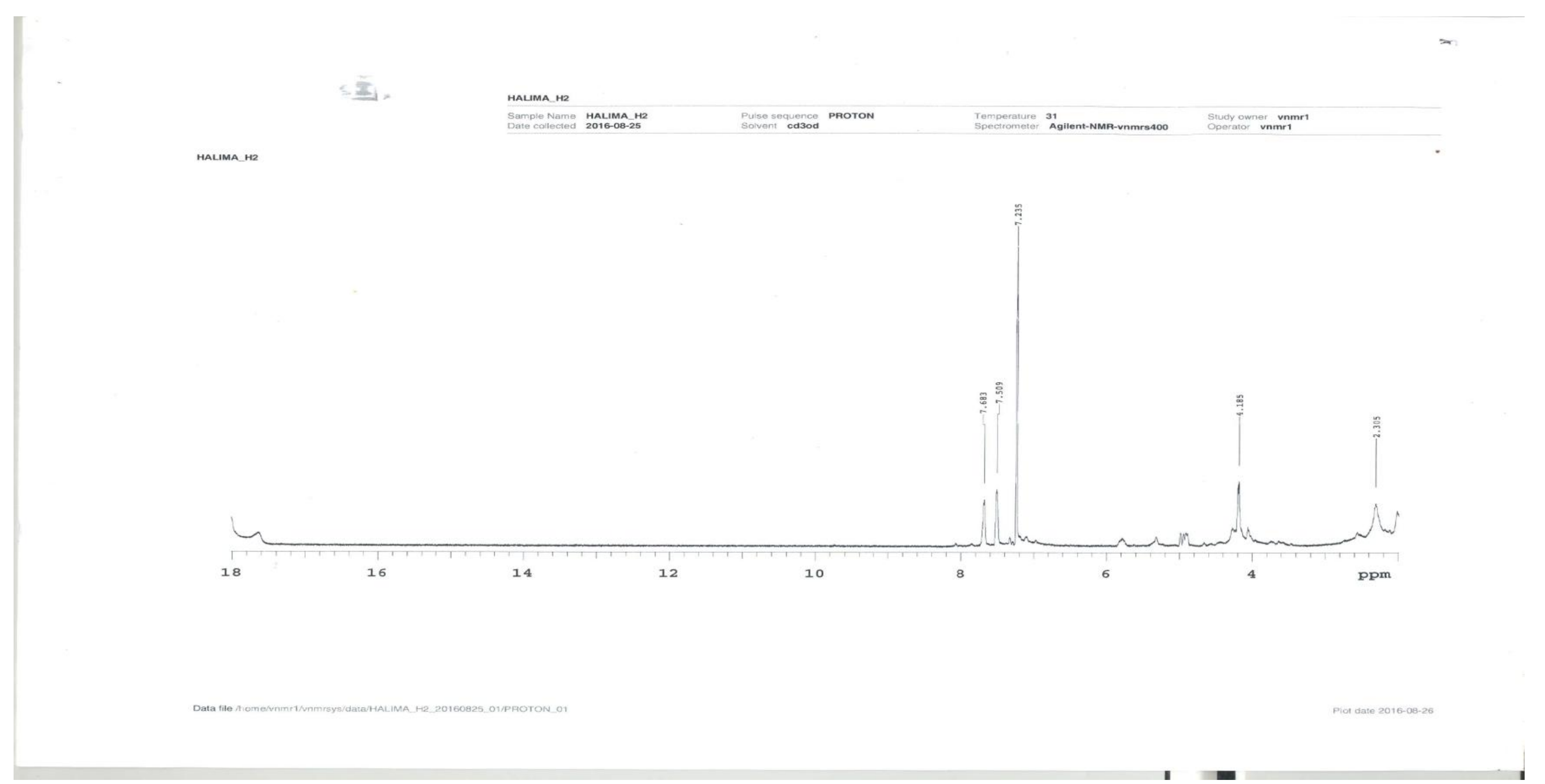
Appendix VII

Thin Layer Chromatography of CPHLE-f1 Sub fraction 3



Appendix VIII

Spectrum of $_1$H NMR of *Carica papaya* hexane leaf extract fraction 1 subfraction 3 (fraction 2)



Appendix IX

Spectrum of $_{13}$C NMR of *Carica papaya* hexane leaf extract fraction 1 subfraction 3 (fraction 2)