LARVICIDAL POTENTIAL OF EXTRACTS OF *Persea americana* SEED AND *Chromolaena odorata* LEAF AGAINST *Aedes vittatus* MOSQUITO

BY

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DECLARATION

I declare that the work in this Thesis titled "Larvicidal Potential of Extracts of *Persea americana* Seed and *Chromolaena odorata* Leaf against *Aedes vittatus* Mosquito" has been carried out by me in the Department of Biochemistry, Ahmadu Bello University, Zaria.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented for another degree or diploma at this or any other Institution.

Name of Student	Signature	Date

CERTIFICATION

This thesis titled "LARVICIDAL POTENTIAL OF EXTRACTS OF *Persea americana* SEED and *Chromolaena odorata* LEAF AGAINST *Aedes vittatus* MOSQUITO" by UMAR, Suleiman Albaba meets the regulations governing the award of the degree of M.Sc. Biochemistry of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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Dean, School of Postgraduate Studies _____ Signature _____ Date

DEDICATION

This research thesis is dedicated to my beloved Mother: Hajia Hadiza Ahmad Arab

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ABSTRACT

The larvicidal activity of various solvent (ethanol, ethyl acetate and n-hexane) extracts of *Persea americana* seed and *Chromolaena odorata* leaves against *Aedes vittatus* mosquito was analysed. The most potent solvent (n-hexane) extracts of both plants were fractionated using column chromatography and most effective fractions isolated and identified using Gas Chromatography Mass Spectrometry and Fourier Transform Infrared techniques. Phytochemical screening revealed the presence of steroids, cardiac glycosides and terpenoids in all the extracts. The larvicidal bioassay of *Persea americana* seed gave LC_{50} values of 0.827ppm, 1.799ppm and 2.732ppm for n-hexane, ethanol and ethyl acetate extracts respectively, while, *Chromolaena odorata* leaf extract had LC_{50} values of 1.835ppm, 3.314ppm, and 5.163ppm for n-hexane, ethanol and ethyl acetate respectively. Column chromatographic fractionation of most potent n-hexane (crude) extracts of both plants, showed increased activity in some of the fractions of *Persea americana* (nHPa6) and *Chromolaena odorata* (nHCo6) which showed higher mortality, with LC_{50} values of 0.486ppm and 1.308ppm respectively. GC/MS analysis of components in nHPa6 and nHCo6 showed oleic acid as the most abundant, in fractions of both plants. The FTIR analyses of nHPa6 and nHCo6 showed absorption bands of the functional groups present, which included; alcohol, alkane, alkene, alkyl halide, aldehyde, carboxylic acid and carbonyl ester, thus, supporting the GCMS result. The n-hexane, ethanol and ethyl acetate extracts of *P. americana* seed and *C. odorata* leaves have shown good larvicidal activity and should therefore be further exploited for the control of mosquito larvae.

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Abbreviations

LC = Lethal Concentrations

GCMS = Gas Chromatography Mass Spectrometry

FTIR = Fourier Transform Infra-Red

n-Hexane = Normal Hexane

TLC = Thin Layer Chromatography

DMSO = Dimethyl Sulfoxide

JE = Japanese Encephalitis

WHO = World Health Organisation

Cx = Culex

An = Anopheles

Ae = Aedes

CHIKV = Chikungunya Virus

Bti = Bacillus Thuriengiensis

Bs = Bacillus Sphaericus

DDT = Dichloro-Diphenyl-Trichloroethane

IGR = Insect Growth Regulator

CSI = Chitin Synthesis Inhibitor

CNS = Central Nervous System

ACh = Acetyl Choline

AChE = Acetyl Choline Esterase

GABA = Gammaamino Butyric Acid

ATP = Adenosine Triphosphate

PTTH = Prothoracictropic Hormone

CCl_4 = Tetrachloromethane

NMR = Nuclear Magnetic Resonance

CHAPTER ONE

1.0 INTRODUCTION

Insect-transmitted diseases remain a major cause of morbidity and mortality worldwide. Mosquito species belonging to genera; *Anopheles*, *Aedes* and *Culex*, are vectors (Redwane *et al*., 2002) for the transmission of malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis (JE), transmitting diseases to more than 700 million people annually (Oyewole *et al*., 2010; Govindarajan, 2009). Mosquitoes also cause allergic responses in humans which include local skin irritation and systemic reactions such as angioedema. *Aedes spp* are generally regarded as a vector responsible for transmission of yellow fever and dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa, Central and South America.

The World Health Organization (W.H.O. 2012) has recommended vector control as an important component of the global strategy for preventing insect-transmitted diseases. The most commonly employed method for the control of mosquito-borne diseases involve the use of chemical-based insecticide, though it is not without numerous challenges, such as human and environmental toxicity, resistance, affordability and availability (Ghosh *et al*., 2012).

Extracts from plants has been good sources of phytochemicals as mosquito egg and larval control agents, since they constitute an abundant source of bioactive compounds that are easily biodegradable into non-toxic products. In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae. They act as larvicides, insect growth regulators, repellents, and oviposition attractants (Pushpanathan, 2008; Samidurai *et al.,* 2009; Mathivanant *et al.,* 2010).

Persea Americana is an ever green tree belonging to *Lauraceae* family and its fruits are commonly known as avocado pear or alligator pear. The plant originates from Central America but it has shown easy adaptation to other tropical regions, thus widely cultivated in tropical and subtropical regions. The various parts (leaves, fruits and seed) of this plant have numerous uses from edible pulp as source of nutrients to the seed preparation as remedy (Arukwe *et al.,* 2012).

The seed extracts of *Persea americana* has many vital application in traditional medicine, for the treatment of diarrhoea, dysentery, tooth ache, intestinal parasites, skin infection (mycoses) and management of hypertension and the leaves have been reported to have anti-

inflammatory and analgesic activities (Adeyemi *et al.,* 2002; Ozolua *et al.,* 2009). Phytochemical screening of avocado seed shows the presence of fatty acids, Triterpenes, anthocyanin, flavonoids and abcissic acids (Leiti *et al.,* 2009).

Chromolaena odorata is a weed which belongs to *Asteraceae* family. It is found in tropical and subtropical areas, extending from west, central and southern Africa to India, Sri Lanka, Bangladesh, Laos, Cambodia, Thailand, southern China, Taiwan, and Indonesia. The weed goes by many common names including; Siam weed, devil weed, French weed, communist weed (Vaisakh and Pandey, 2012). In Nigeria, the *Chromoleana odorata* is referred to as "*Obu inenawa*" by the Igbo and "*ewe awolowo*" by the Yoruba. This plant is exploited traditionally for its medicinal properties, especially for external uses as in wounds, inflammation and skin infections. Some studies also demonstrate the efficacy of its leaf extract, as antioxidant, anti-inflammatory, analgesic, anti-microbial and cytoprotective agent (Ajao *et al.,* 2011). The oil from *C. odorata* also had been exploited as insecticide, ovicide and larvicide (Noudogbessi *et al.,* 2006). Previous phytochemical studies of the leaf extracts of *C. odorata* show the presence of alkaloid, cardiac glycosides, anthocyanin, tannin, and flavonoids (Ngozi *et al*., 2009).

1.1 Statement of Research Problem

An estimated 3.3 billion people are at risk of malaria globally, with populations living in sub-Saharan Africa having the highest risk (WHO, 2012) and two-fifths of the world's population is at risk of dengue fever (WHO, 2003). Malaria alone accounts for about 50 per cent of out-patient consultation, 15 per cent of hospital admission, and also among the top three causes of death in the country.

In recent years, the use of many synthetic insecticides in mosquito control programme has been limited, due to many challenges such as, high cost of synthetic insecticides, environmental sustainability, toxic effect on human health (immune suppression), and other non-target organisms, environmental persistence, higher rate of biological accumulation and magnification through ecosystem, as well as increasing insecticide resistance on large scale (Srivastava and Sharma, 2000; Raghvendra and Subbarao, 2002). These challenges have resulted in an urge to search for environmentally sustainable, biodegradable, affordable and target selective insecticides against mosquito species (Saxena and Sumithra, 1985; Kumar and Dutta, 1987; Chariandy *et al*., 1999; Markouk *et al*., 2000; Tare *et al*., 2004).

Consequently, the application of eco-sustainable alternatives such as biological control of vectors has become the main focus of the control programme to replace the synthetic chemical insecticides (Gosh *et al.,* 2012). One of the most effective alternative approaches under the biological control programme is to utilise the plants biodiversity as a reservoir of safer insecticides of botanical origin as a simple, affordable and sustainable method of mosquito control.

1.2 Justification

Mosquito larvae is the easiest stage to target in its life cycle and several studies have documented the efficacy of plant extracts as a reservoir pool of bioactive toxic agents against mosquito larvae. Furthermore, evolution of the resistance to plant-derived compounds has rarely been reported (Sharma *et al.,* 2006).

However, the main reasons for the failure in laboratory to field utilisation of bioactive phytochemicals are poor characterization and inability to determine the active toxic components responsible for larvicidal activity (Ghosh *et al.,* 2012). Hence, there is a need for the characterisation, of various plant extracts to determine the active (larvicidal) components of locally available plants for mosquito control. This will help to reduce dependence on expensive and mostly imported products, and stimulate local efforts to enhance the general public health.

1.3 Aims and Objectives

1.3.1 General Aim

The aim of this study was to investigate the larvicidal potential of extracts of *Persea americana* seed and *Chromolaena odorata* leave against *Aedes vittatus* larvae

1.3.2 Specific Objectives

a. Phytochemical analysis (qualitative) of the crude extracts of *persea americana* seed and *Chromolaena odorata* leaves.

b. Determination of the most potent solvent extracts with larvicidal activity against *Aedes vittatus* larvae

c. Determination of the lethal concentration (LC) of the crude extracts for 50% and 90% mortality (LC_{50} and LC_{90}).

d. Fractionation of the most potent crude extracts and isolation of the most effective (larvicidal) fractions using column chromatography;

e. Characterisation of the bioactive (larvicidal) fractions using FTIR and GC/MS techniques.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mosquito

Mosquitoes are members of a family of the *Culicidae*, from the Latin word *culex*, meaning "midge" or "gnat". The word mosquito is from the Spanish or Portuguese for "little fly" (Ralph, 2008). They are small, two winged insects belonging to *Culicidae* family and order *Diptera* (two winged flies) (Muesebeck, 1952; Goma, 1966). Nearly three quarters of all mosquito species are found in the humid tropics and subtropics (Miyagi *et al.,* 1992).

Mosquitoes are among the best known groups of insects, because of their medical importance to man as pests and vectors of some of the most deadly human diseases. There are over 3,500 species of mosquitoes described from various parts of the world. The three subfamilies recognised among the *Culicidae* family are: the *Toxorhynchitinae, Anophelinae* and *Culicinae*. Subfamily *Toxorhynchitinae* comprises a single genus, *Toxorhynchites*, which is not of importance medically. There are 3 genera in subfamily *Anophelinae*, but only *Anopheles* is of medical importance. There about 60 species of *Anopheles* mosquitoes known to be vectors of malaria. Members of subfamily *Culicinae* that are of medical importance include; *Culex*, *Aedes*, *Mansonia*, *Haemagogus* and *Sabethes*. There are over 2500 species of *Culicinae* of which the main genera are *Aedes* with more than 900 species. *Aedes* is best known vectors of yellow fever and dengue fever but some *Aedes* species double as vectors of some filariasis and viral disease (Leisnham, 2010).

2.1.1 Classification of *Aedes vittatus*

Kingdom: *Animalia*

Phylum: *Arthropoda*

Class: *Insecta*

Order: *Dipte*ra

Family: *Culicidae*

Subfamily: *Culicinae*

Genus: *Aedes*

Species: *vittatus*; Binomial: *Aedes vittatus*

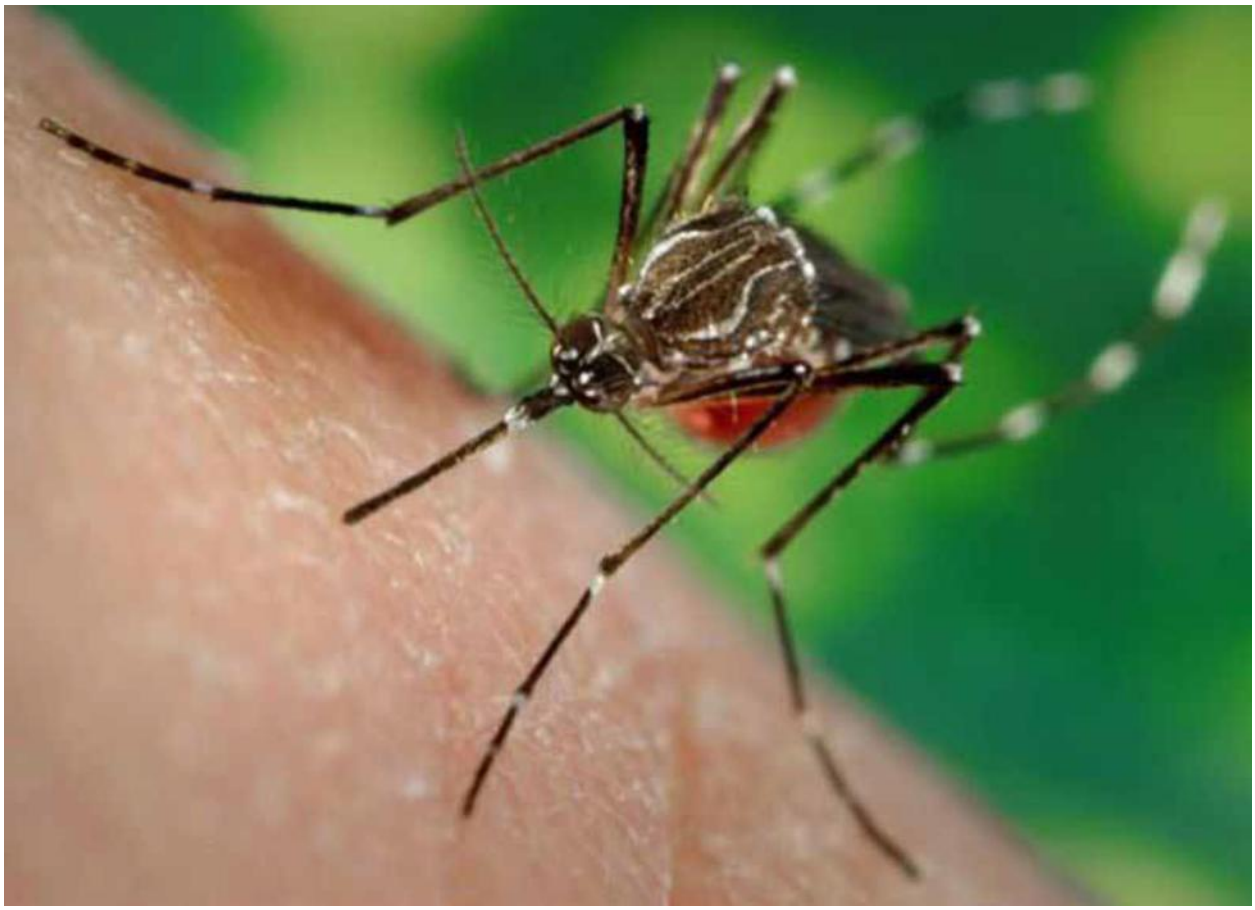


Plate 2.1: *Aedes* Mosquito

Source: U.S.A. Environmental Protection Agency (2007)

2.1.2 Distribution and Dispersal of Mosquitoes

Mosquitoes are widespread and occur in all regions of the world except for Antarctica (Mullen and Durden, 2009). In warm and humid tropical regions, they are active for the whole year, whereas in temperate regions they usually hibernate over winter. Arctic mosquitoes may be active for only a few weeks as pools of water form on top of the permafrost (Fang, 2010). Eggs from strains in the temperate zones are more tolerant to the cold than ones from warmer regions. They can even tolerate snow and sub-zero temperatures. In addition, adults can survive throughout winter in suitable microhabitats (Hawley, *et al*., 1989; Hanson and Craig, 1995).

Worldwide introduction of various mosquito species over large distances into regions where they are not indigenous has occurred through human activities, primarily on sea routes, in which the eggs, larvae, and pupae inhabiting water-filled used tires and cut flowers are transported. However, apart from sea transport, mosquitoes have been effectively carried by personal vehicles, delivery trucks, trains and aircraft. Sufficient quarantine measures have proven difficult to implement (Romi *et al*., 2006).

2.1.3 Life Cycle of *Aedes*

Mosquitoes like all flies go through four stages in their life cycle, which include; egg, larva, pupa, and adult. However, each stage in the mosquito life cycle has diverse morphology. They complete their lifecycle both in aquatic and terrestrial habitats, but different species have different ecological adaptations with respect to where they lay their eggs. The first three stages; egg, larva and pupa—are aquatic and the duration of these stages may vary, depending on the species, food resources and the ambient temperature.

2.1.3.1 Egg stage

Mosquitoes prefer to lay their eggs on water sheltered from the wind by grass and weeds. The oviposition and morphology of eggs vary considerably between species (Huang *et al.*, 2006). *Aedes* females usually drop their eggs singly on damp mud or other surfaces near the water edge that is easily flooded or washed into the water (Kosova, 2003). The eggs generally do not hatch until they are flooded, and they can withstand considerable desiccation before that happens (Spielman and D'Antonio, 2001). They are not resistant to desiccation immediately after oviposition, but must develop to a suitable degree first. *Aedes* batch of eggs tend to

hatch irregularly over an extended period of time which makes it much more difficult to control such species than those mosquitoes whose larvae can be killed all together as they hatch (Hanson and Craig, 1995).

2.1.3.2 Larva Stage

Mosquito larvae are commonly called "wigglers" or “wrigglers” owing to their mode of movement in water where they swim in an s-shaped or vermiform pattern and usually dive to the bottom when the water surface is suddenly disturbed. The larval stage displays a great variation in morphology between the mosquito species and can be used for identification purposes. *Aedes* larvae need to breathe atmospheric oxygen and therefore lay 45 degrees to the water surface (Romi *et al*., 2006). They feed on organic matter and micro-organisms such as algae and bacteria, in the water. Mosquito larvae undergo four larval stages called instars (period between successive moulting increasing in size following each moulting) that require five to ten days to complete. The variation of duration depends on temperature (WHO, 1973b) or larval diets (Hawley, 1989). When the 4th instar larva moults it becomes a pupa.

2.1.3.3 Pupa Stage

This is the transition stage between the aquatic stages of the mosquito’s lifecycle and the terrestrial adult stage. It is comma shaped and use tumbling movement to escape predation, thus, are commonly called “tumblers”. If undisturbed, pupae float at the surface and breathe oxygen through two breathing tubes called "trumpets" located on the cephalothorax. Pupae do not feed, and therefore, maintain low activity. The metamorphosis of the mosquito into an adult is completed within the pupal case (Spielman, 2001). This is achieved when the dorsal surface of its cephalothorax splits, and the adult mosquito emerges which rests on water surface until its body hardened.

2.1.3.4 Adult Stage

After the adult mosquito emerges, it seeks a protective environment in the surrounding vegetation to allow its wings to complete development. Male mosquitoes tend to emerge prior to the female mosquito and will mate with the female as soon as she is able. Female and male mosquitoes require carbohydrate such as plant nectar and other sources of sugar throughout their life to maintain energy for flying, mating, and seeking hosts for blood meals. However, only the female mosquito takes a blood meal because of extra protein to develop eggs. During

the process of taking a blood meal, the mosquito is able to transmit viruses, *protozoan*s, and *helminthes* to humans and other animals. After obtaining a blood meal, the female will rest for a few days while the blood is being digested and eggs are developing. When the eggs are fully developed, the female lays them and continue with host-seeking (Spielman, 2001).

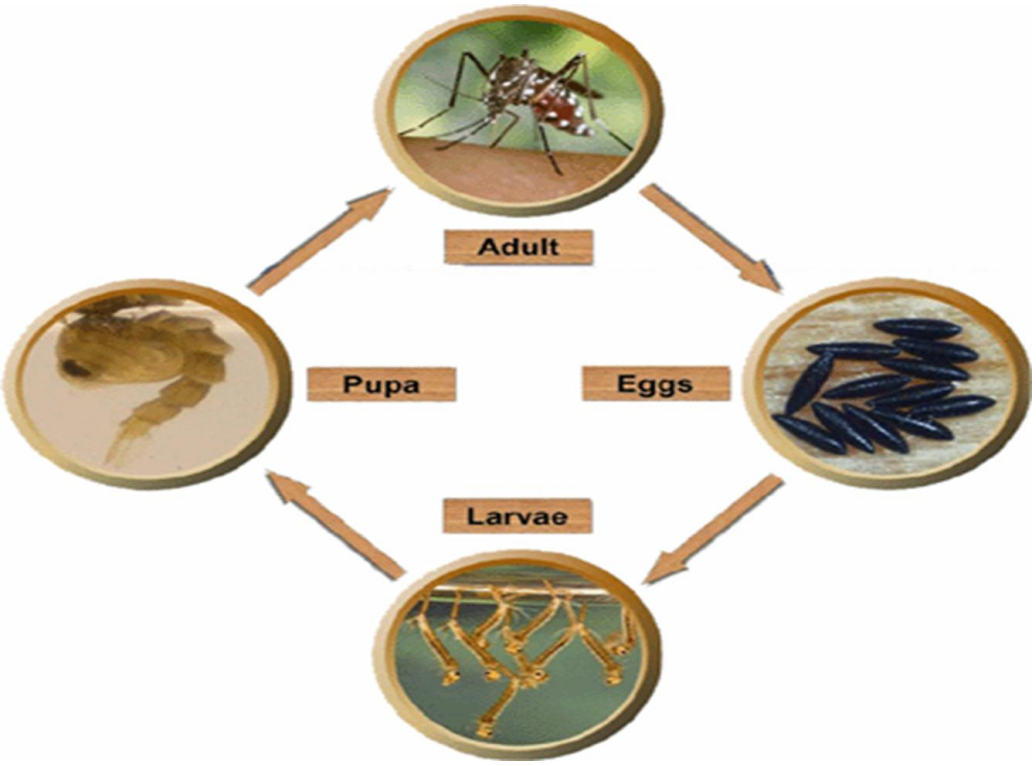


Figure 2.2: Life Cycle of Aedes mosquito

Source: U.S.A. Environmental Protection Agency (2007)

2.1.4 Mosquito Morphology and Feeding Habits

Mosquitoes feed on nectar and plant juices, but the mouthparts of the blood sucking females are suitable for piercing the skin of animal hosts as their ectoparasites. In many species, the female needs to obtain nutrients from a blood meal in order to produce eggs. Autogenous reproduction is said to occur when female reproduces without such parasitic meals, as in *Toxorhynchites*; otherwise, the reproduction may be termed anautogenous, as occurs in mosquito species that serve as disease vectors, particularly *Anopheles* and some of the most important disease vectors in the genus *Aedes*. However, some mosquitoes, such as *Culex*, are partially anautogenous because they do not need a blood meal for their first cycle of egg production, which they produce autogenously but subsequent clutches of eggs are produced anautogenously, during which their disease vectoring activity becomes effective (Sawabe and Moribayashi, 2000).

Mosquitoes, irrespective of species have slender bodies with three segments which include: head, thorax and abdomen. The head is specialized for sensory information reception and feeding and has compound eyes with a pair of long segmented antennae. The compound eyes are separated from one another. The antennae are important for detecting odour of their host and breeding site (Harzsch and Hafner, 2006). Another feature on the head is an elongated, forward-projecting "stinger-like" proboscis used for feeding, and two sensory palps.

The thorax is adapted for locomotion with three pairs of legs and a pair of wings attached to it. The abdomen is specialized for food digestion and egg development; the abdomen of a mosquito can hold three times its own weight in blood. Abdominal segment expands considerably when a female takes a blood meal. The blood is digested over time, which serves as a source of protein essential for the production of eggs that gradually fill the abdomen.

Proboscis is the most vital feeding structure of the mosquito and labium is the visible part of the proboscis which forms the sheath enclosing the two mandibles, two maxillae, the hypopharynx, and the labrum, mandibles and the maxillae are used for piercing the skin (Wahid *et al.*, 2003). When the mosquito first lands on a potential host, the mouthparts will be enclosed entirely in this sheath, which touch the tip of the labium to the skin in various places. Mosquito wander and prod around, apparently looking for a suitable place for a considerable time before biting. Presumably, this probing is a search for a place with easily accessible blood vessels, but the exact mechanism is not known. It is known that there are

two taste receptors at the tip of the labium, which may well play a role (Mullen and Durden, 2009). The mosquito moves its head backwards and forwards to force mouthparts into the skin of its host with the maxillae and mandibles opposing movement. Hypopharynx and the labrum are hollow in which saliva with anticoagulant is pumped down the hypopharynx to prevent blood clotting, while blood is drawn up the labrum. The mechanisms effectively block the hemostasis system with a mixture of secreted proteins in the saliva which negatively affects vascular constriction, blood clotting, platelet aggregation, angiogenesis and immune response leading to inflammation (Ribeiro and Francischetti, 2003). In addition to anticoagulants, mosquito saliva contains sugar digesting enzymes and antimicrobial agents to aid sugar feeding and control bacterial growth in the sugar meal (Rossignol and Lueders, 1986; Grossman and James, 1993). The composition of mosquito saliva contains less than 20 dominant proteins which have an ability to modulate the immune response of their hosts (Valenzuela *et al.,* 2002). However, the mechanism for mosquito saliva-induced alteration of the host immune response is unknown (Ribeiro, and Francischetti, 2003).

2.1.5 Mosquito Born Diseases

Many species of mosquitoes are estimated to vector various types of disease to over 700 million people in Africa, South America, Central America, Mexico, Russia and many parts of Asia, resulting in the death of, at least two million people per annum. Mosquitoes carry these disease-causing viruses and parasites and transfer them from person to person without showing symptoms of the diseases (Tolle, 2009). Mosquito-borne diseases include: Malaria, Filariasis, Japanese Encephalitis, Chikungunya, West Nile Virus, Tularemia, Rift Valley Fever, Yellow Fever, and Dengue Fever.

2.1.5.1 Malaria

Malaria is a parasitic disease caused by five species of the *Plasmodium* parasite. The species affecting humans include: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. The most deadly form of the malaria parasite predominates in Africa which is due to *P. falciparum*; while *P. vivax* is less dangerous but more widespread, and the other three species are less frequently found in Africa (Collins, 2012). The parasites are transmitted from bites of an infected female *Anopheles* mosquito to man and *Culex* to avians.

It is estimated that, 3.3 billion people in about 107 countries and territories were at risk of malaria in 2011, with sub-Sahara African populations having the highest risk of malaria infection, of these, 80% of cases and 90% of mortality occur in WHO African Region, with pregnant women, children under the age of five years, sickle cell anaemia and HIV/AIDS patients are most severely affected (WHO, 2012. In Nigeria, malaria accounts for over 50% of all out-patient consultation and 30% of hospital admissions. It is responsible for 29% of childhood death, 25% of infant mortality and 11% of maternal mortality (Alaba, 2005).

Nigeria is among countries where malaria is responsible for an estimated average annual reduction of 1.3% in economic growth equivalent to N132 Billion annually (Alaba, 2005; Roll Back Malaria Programme, 2000). The burden of this disease has been a major source of concern to government and development partners (malaria endemic countries, multilateral and bilateral donors, nongovernmental organizations, civil society, academia, and private organizations) which has been expressed through many instruments such as Abuja Declaration in year 2000, Millennium Development Goals to reduce disease burden by 2015 and Universal Access to HIV/AIDS, tuberculosis and Malaria in 2006 (WHO, 2003).

2.1.5.2 Filariasis

Filariasis (philariasis) is an infectious tropical disease affecting both human and domestic animals. It is caused by thread-like parasitic nematodes that belong to the superfamily *Filarioidea*. The parasitic species include *Wuchereria bancrofti*, *Brugia malayi*, *Brugia timori* and they are transmitted from host to host by blood-sucking arthropods, mainly black flies and mosquitoes (Ottesen *et al*., 2008). These worms occupy the lymphatic system, including the lymph nodes, in chronic cases, these worms lead to the disease elephantiasis which is the most spectacular symptom of lymphatic filariasis characterised by oedema with thickening of the skin and underlying tissues. Filariasis is considered endemic in tropical and subtropical regions of Asia, Africa, Central and South America, and Pacific Island nations, with over 120 million people infected and a billion people at risk of infection. The disease is endemic in 73 countries, out of which, 37 are in Africa. (Hopkins, 2013)

Human filarial nematode worm lifecycles consists of five stages. After mating, the female gives birth to thousands of live microfilariae. During a blood meal, the microfilariae are taken up by the vector insects as intermediate host where it moults and develop into third-stage (infective) larvae. In subsequent blood meal, the vector insect injects the infectious larvae

into the dermis layer of the skin. After about one year, the larvae moult through two more stages, maturing into the adult worms (Outland, 2005).

The strategy for eliminating transmission of lymphatic filariasis is mass distribution of medicines that kill the microfilariae and stop transmission of the parasite by mosquitoes in endemic communities. In sub-Saharan Africa, albendazole is being used with ivermectin to treat the disease. Prevention of mosquito bites by using insecticide-treated mosquito bed nets, also reduces the transmission of lymphatic filariasis.

2.1.5.3 Japanese Encephalitis

This is a viral disease caused by the mosquito-borne Japanese encephalitis virus. The Japanese encephalitis virus is a virus from the family *Flaviviridae* (Nimesh and Lakshmana, 2011). Domestic pigs and wild birds (herons) are reservoirs of the virus. The most important vectors of this disease are the mosquitoes *Culex tritaeniorhynchus* and *Culex vishnui* (Kim *et al.*, 2011). Human, cattle and horses are dead-end hosts and disease manifests as fatal encephalitis. Japanese encephalitis (JE) is the leading cause of viral encephalitis in Asia, with 30,000–50,000 cases reported per annum and most prevalent in Southeast Asia and the Far East. Infection with JEV confers lifelong immunity but there is no specific treatment for Japanese encephalitis and treatment is supportive, with assistance given for feeding, breathing or seizure control as required (Solomon, 2006).

2.1.5.3 Chikungunya

Chikungunya means "that which bends up", in Makonde language. Chikungunya virus belongs to alphavirus genus of the *Togaviridae* family. It is an arthropod-borne virus that is transmitted to humans by virus-carrying *Aedes* mosquitoes (Lahariya and Pradhan, 2006).

CHIKV infection causes an illness which affects the joints of the extremities (Powers and Logue, 2007). The pain associated with CHIKV infection of the joints persists for weeks or months, or in some cases years. Other symptoms include intense headache, insomnia, nausea, vomiting, conjunctivitis, slight photophobia and partial loss of appetite. The disease is prevalent in Africa, South Asia, and Southeast Asia, (Chahabra *et al.*, 2008).

The most effective means of prevention are protection against contact with the disease-carrying mosquitoes through mosquito control because there are no specific treatments for chikungunya, and no vaccine is currently available.

2.1.5.4 Yellow Fever

Yellow fever, also known as Yellow Jack, is an acute viral haemorrhagic disease caused by yellow fever virus that is transmitted by an infected female mosquitoes, *Aedes* species mostly found in tropical and subtropical regions of South America and Africa (McNeill, 2004). The only known hosts of the virus are human, other primates and several species of mosquito. The symptoms of the disease include; fever, nausea, anorexia, muscle pain, backache and headache (Finlay, 2012).

The World Health Organization estimates that yellow fever causes 200,000 illnesses and 30,000 deaths per annum in unvaccinated populations. Although, the number of officially reported cases is far lower, nearly 90% of the infections occur in Africa (Mutebi and Barrett, 2012). There are three epidemiologically different infectious cycles in which the virus is transmitted from mosquitoes to humans or other primates, these include: Sylvatic Cycle, Urban Cycle and Savannah Cycle (Barrett and Higgs, 2007). The urban cycle is responsible for the major outbreaks of yellow fever that occur in Africa. In Urban Cycle only the yellow fever mosquito *Aedes aegypti* is involved. There exist in Africa and South America, a Sylvatic Cycle (forest cycle or jungle cycle), where *Aedes africanus* (in Africa) or mosquitoes of the genus *Haemagogus* and *Sabethes* (in South America) serve as vectors. In the jungle, the mosquitoes infect mainly non-human primates; the disease is mostly asymptomatic in African primates. Savannah Cycle occurs between the jungle and urban cycle with different mosquitoes of the genus *Aedes* involved. In recent years, this has been the most common form of yellow fever transmission in Africa Clememt, 1992.

A safe and effective vaccine against yellow fever has existed since the middle of the 20th century, and some countries require vaccinations for travellers. Because no therapy is known, vaccination programs are of great importance in affected areas coupled with measures to prevent mosquito bites and reduce the population of the mosquito transmitting the virus Tolle, 2009.

2.1.5.4 Dengue Fever

This is an infectious tropical disease caused by dengue virus which is transmitted by several species of mosquito within the genus *Aedes*. The *Aedes* species that transmit the disease include *A. aegypti*, *A. albopictus*, *A. polynesiensis* and *A. scutellaris*. A female mosquito that takes a blood meal from a person infected with dengue fever becomes infected with the virus

in the cells lining its gut. About 8–10 days later, the virus spreads to other tissues including the mosquito's salivary glands and is subsequently released into its saliva.

Dengue is endemic in more than 110 countries, affecting 50 to 390 million people and leads to half a million hospital admissions with about 25,000 deaths worldwide annually (Whitehorn and Farrar, 2010; Bhatt *et al.,* 2013). It is the most common viral disease transmitted by arthropods, with a disease burden of about 1600 disability-adjusted life years per million population, which is similar to tuberculosis, another childhood and tropical disease (Rodenhuis-Zybert *et al.,* 2010). Dengue disease was deemed second in importance to malaria in 1998, though the World Health Organization counts dengue as one of seventeen neglected tropical diseases (Guzman *et al*., 2010). Like most arboviruses, dengue virus is maintained in nature in cycles that involve preferred blood-sucking vectors and vertebrate hosts (Gubler, 2010). The viruses are maintained in the forests of Southeast Asia and Africa by transmission from female *Aedes* mosquitoes—of species other than *A. aegypti*—to her offspring and to lower primates (Wiwanitkit, 2010; Simmons *et al.,* 2012). There are no vaccines so far approved for the dengue virus. Prevention therefore depends on control of the mosquitoes transmitting the disease (Whitehorn and Farrar, 2010).

2.1.5.5 Mosquito Bites and Treatment

Mosquito bites usually result to irritation and inflammation on the skin (spot bitten) due to an immune response elicited by binding of host’s (human) IgG and IgE antibodies to antigens present in the mosquito's saliva. Some of the sensitizing antigens are common to all mosquito species, whereas others are specific to certain species. There are both immediate hypersensitivity reactions and delayed hypersensitivity reactions to mosquito bites (Higgs, 2004). Both reactions result in itching, redness and swelling. Immediate reactions develop within a few minutes of the bite and last for a few hours. Delayed reactions take around a day to develop, and last for up to a week. Several anti-itch medications are available, such as Benadryl, or topically applied antihistamines and, for more severe cases, corticosteroids, such as hydrocortisone and triamcinolone. Tea tree oil has been shown to be an effective anti-inflammatory, reducing itching (Clements, 1992).

2.1.6 Mosquito Control Methods

Many methods used for mosquito control depend on the situation but the most important and employed methods include: source reduction (e.g., removing stagnant water, modification of breeding site); biocontrol (e.g. use of natural predators such as dragonflies, biological agents: bacteria, fungi,); use of chemical (e.g. Insecticides to kill larvae or adults); exclusion (mosquito nets and window screening)

2.1.6.1 Source Reduction

Source reduction, also known as physical or permanent control, means elimination of breeding places of mosquitoes by engineering measures such as filling, levelling and drainage of breeding sites and water management (such as intermittent irrigation). While it can be possible to fill small artificial ponds that produce mosquitoes, it is usually impossible to do so in natural areas (however small) large permanent water bodies, or in areas set aside for storm water or wastewater retention.

Source reduction can also be done by making water unsuitable for mosquitoes to breed, for example, modification of breeding site by using surface active agent and changing salinity of water has been practiced and found to be effective, though not without effects on the ecosystem.

The surface active agents are non-ionic, biologically degradable substances which spread spontaneously to form a monomolecular film on the water surface when applied to mosquito breeding habitats and exhibits mortality of mosquito larvae. Surface active agents have been used effectively to control *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti* in different breeding habitats such as cesspits, cesspools, drains and wells (Das *et al.*, 1986).

2.1.6.2 Exclusion

Window screens and mosquito nets are the most effective measures for residential areas. Insecticide-impregnated mosquito nets are particularly effective because they selectively kill those insects that attack humans, without affecting the general ecology of the area.

2.1.6.3 Biological Control

The use of biological agents to control mosquitoes is highly specific with limited non-target or environmental effects and can be used to control immature as well as adult mosquitoes. Dragonfly and damselfly nymphs eat mosquitoes at various stages of development and can be useful in reducing mosquito population (Singh *et al*., 2003). Insectivorous fishes, such as

species of *Galaxias* and members of the *Poeciliidae*, e.g. Gambusia and guppies (Poecilia), eat mosquito larvae and sometimes are introduced into ponds to assist in control. Larvivorous fishes were the first biocontrol agents employed to control mosquito and have been used in many countries for malaria control e.g. *Oreochromis spilurus* was found to be effective against malaria vectors in Somalia (Mohammed, 2003). Predatory mosquito, *Toxorhyncites splendens* is a non-blood sucking mosquito whose larvae were found to be effective in controlling *Anopheline* and *Culicine* larvae by feeding on them (Amalraj and Das, 1998) and efficacy of this mosquito was proved in field evaluation studies against thirteen species of mosquitoes (Miyagi *et al*., 1992).

Bacillus thuringiensis (*Bti*) and *Bacillus sphaericus* (*Bs*) are entomopathogenic and spore forming bacteria, producing upon sporulation a parasporal crystal (protein) toxic to some invertebrates, mostly insects and nematodes (Feitelson *et al.,* 1992). *Bti* is toxic to both mosquito and blackfly whereas *Bs* only to mosquitoes. The larvicidal properties of the crystal have made *Bs* a useful agent for the biological control of mosquitoes. Other bacteria have also been found to be highly toxic to mosquito larvae, including *Clostridium bifermentans* (Poopathi and Baskaran, 2001). These bacterial species although pathogenic to the target vector species, are non-hazardous to other beneficial and non-target organisms. Bacterial mosquito larvicides may however, suffer the same fate in inducing resistance development as synthetic insecticides.

Other mosquito biological control agents include Cyclopoid copepods like *Macrocyclops distictus, Mesocyclops pehpeiensis* and *Megacyclops viridis* used as control agents against dengue vector, *Aedes albopictus* in Japan (Dieng *et al.,* 2002); many nematodes of the genus *Romanomermis* such as *R.iyengari* and *R.culicivorax* have a broad host range and are promising bio control agents of various species of mosquitoes (Petersen, 1982, 1985; Rodriguez *et al*., 2003). Several viruses such as *Iridescent virus*, *Densonucleosis virus*, *Cytoplasmic polyhedrosis virus*, *Nuclear polyhedrosis virus* etc. have been evaluated in the past for mosquito control (Jenkins, 1964; Roberts and Strand, 1977). Many fungi such as *Coelomomyces*, *Lagenidium*, *Metarrhizium*, *Culinomyces* and *Tolypocladium* have been isolated and tested for mosquito control (Roberts and Strand, 1977). *Metarhizium anisopliae*, was found to be effective against *Anopheles gambiae* and *Cx. quinquefasciatus* (Lopez *et al.,* 2004).

2.1.6.4 Chemical Control Method

Many synthetic chemicals are used as insecticides to prevent and control insect pest at various developmental stages. The most commonly used group of chemicals include; Organophosphates, carbamates and organochlorides which are broad-spectrum insecticides with relatively higher toxicity and longer residual activities (Foster and Walker 2002). Many organophosphates such as Chlorpyrifos and Malathion can be used as larvicides or outdoor residual sprays for mosquito control. However, many organophosphates, in particular Chlorpyrifos, are highly toxic to fishes (O'Neill, 1997).

Larvicides are applied to water where mosquito larvae develop or where it may provide a habitat for mosquitoes. Currently, light mineral oils and insect-growth regulators such as temephos are used as mosquito larvicides in many countries. Insect growth regulator (IGR) retards the development of larvae and prevents mosquitoes from developing into adults e.g. Methoprene. Larvae treated with this chemical fail to moult successfully and simply die during the pupal stage. The target-specific S-Methoprene will not affect fish, waterfowl, mammals or the environment when applied at the recommended dosage (O'Neill, 1997).

Synthetic pyrethroids are derivatives of the natural Pyrethrins extracted from *Chrysanthemum* flowers. Pyrethroids such as S-Bioallethrin, Cypermethrin, Permethrin, Etofenprox and Cyfluthrin are broad-spectrum contact insecticides that provide rapid knockdown effects on insects but with only little residual activity. Chemical insecticides are applied to target pests in a myriad of formulations and delivery systems e.g sprays, baits, slow-release diffusion, (Ware and Whitacre, 2004).

The problems associated with the use of chemical insecticides include; affordability and availability, concern for environment, harmful effects on human health and non-target species, their non-biodegradable nature, biological magnification through ecosystem and

resistance development (WHO, 1996; Brown, 1986). These problems stimulated researchers to look for cost-effective, environment friendly, biodegradable, target selective and irresistible insecticides against mosquito species. Consequently, scientists explore the floral biodiversity and search for safer insecticides of plant origin as a simple, sustainable and efficient, with very little chance of mosquitoes developing resistance, as well as suitable and adaptive to ecological conditions.

2.1.6.5 Use of phytochemicals for mosquito control

Phytochemicals are botanicals which occur naturally and are basically secondary metabolites that serve as a means of defence mechanism of the plants to withstand the continuous selection pressure from herbivore predators and other environmental factors. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities (Shaalan *et al.*, 2005).

Many African local communities employ various methods to repel mosquitoes traditionally using different plant parts. Application of smoke by burning the plant parts is one of the most common practices. Other types of applications are spraying the extracts of the repellent plant parts, hanging and sprinkling the repellent plant leaves on the floor. The leaf of plant is one of the most commonly and extensively used plant parts to repel the insects and mosquitoes, followed by root, flower and remaining parts of repellent plants (Karunamoorthi *et al.*, 2009,).

Approximately 1,200 plant species with potential insecticidal value were described by Roark 1947, while 344 plant species that only exhibited mosquitocidal activity were listed and discussed (Sukumar, *et al.* 1991). According to Ghosh *et al*., (2012); more than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control

programmes with members of the plant families-*Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae* as most studied having various types of larvicidal, adulticidal or repellent activities against many species of mosquitoes. Shaalan *et al.,* (2005) reviewed the current state of knowledge on larvicidal plant species, extraction processes, growth and reproduction inhibiting phytochemicals, botanical ovicides, synergistic, additive and antagonistic joint action effects of mixtures, residual capacity, effects on non-target organisms, resistance and screening methodologies, and discussed some promising advances made in phytochemical research.

Application of larvicide from botanical origin was extensively studied and various mosquito control agents such as ocimenone, rotenone, capllin, quassin, thymol, eugenol, neolignans, arborine and goniothalamin were developed (Shaalan, *et al.,* 2005).

2.1.7 Active Ingredients in Plants Responsible for Larval Toxicity

The plant kingdom contains a rich source of phytochemicals that are used in place of synthetic insecticides in mosquito control programme as reviewed by Kishore *et al.,* (2011). Several plants derived chemicals, such as, alkanes, alkenes, alkynes and simple aromatics, lactones, essential oils and fatty acids, terpenes, alkaloids, steroids, isoflavonoids, pterocarpans and lignans were studied and described according to their chemical nature and mosquito larvicidal potentiality. Ghosh *et al.,* (2012); reported that, Octacosane, α-terpinene, Geranial, Germacrene D, Hugorosenone, Azadirachtin, Dioncophylline-A, Methyl-*p*-hydroxybenzoate, β-sitosterol, Pipernonaline are some of the active ingredients isolated from many plants species and studied for larvicidal activity against different species of mosquitoes. Of these phytochemicals; 4-ethoxymethylphenol, 4-butoxymethylphenol, vanillin, 4-hydroxy-2-methoxycinnamaldehyde, and 3,4-dihydroxyphenylacetic acid isolated from *Vanilla fragrans* show 100% mortality potency against mosquito larvae at 0.5, 0.4, 2.0 and 1.0 mg/mL. The lipophilic falcarinol with LC_{50} values of 3.5 and 2.9 ppm in 24 h and 48 h,

respectively, exhibit strong toxicity than the more polar falcarindiol both isolated from *Cryptotaenia canadensis* tested against *Culex pipiens* larvae. The fatty acid constituents, linoleic acid and oleic acid isolated from *Dirca palustris* exhibit mosquitocidal activity against fourth instar *Ae. aegyptii* larvae with LD_{50} values of 100 µg/mL at 24 h, each (Ramsewak *et al.*, 2001)

2.1.8 Mechanism and mode of action of insecticide/larvicide

Mechanism and mode of action describe phenomena at the molecular level which result to functional or anatomical change, at the cellular level, due to the exposure of a living organism to a substance (U.S., Environmental Protection Agency, 2007). A complete understanding of the mode of action of an insecticide requires knowledge of how it affects a specific target site within an organism. This provides understanding of how insecticides work on targeted insects system which may offer explanations with regard to development of resistance to a particular class of insecticide (Goodell, 2004). Insecticides generally target systems and functions ranging from nervous system, growth and development to metabolism and energy production, of these, the primary targets are critical proteins (enzymes, receptors, ion-channels and structural proteins) and hormones (Ghosh *et al.*, 2012).

The nervous system functions as a fast acting means of transmitting and interpreting important information throughout the body. Insect's growth and development depend on their ability to shed their skin in order to reach next stage in their life cycle and energy is essential for all of these processes to take place. Thus, many insecticides act by interfering with these processes which in turn, affects insect physiology in many different ways as explained below:

2.1.8.1 Choline esterase inhibition

Acetyl choline is a neurotransmitter (chemical messenger) that can either excite or inhibit its target neurons – depending on the particular neuron and the specific receptors at the site, ACh

can cause particular neurons to “fire,” continuing the nerve impulse transmission, or it can cause the nerve impulse to stop at that particular site. Thus, acetyl choline esterase is an enzyme that is responsible for breaking down of ACh after it has carried its message across the synapse. Organophosphate and carbamates insecticides bind and inhibit the enzyme, cholinesterase. When an insect has been poisoned by a cholinesterase inhibitor, the cholinesterase is not available to help break down the ACh, and the neurotransmitter continues to cause the neuron to send its electrical charge which causes overstimulation of the nervous system, and the insect dies. Cholinesterase inhibition by carbamates is somewhat reversible while organophosphate poisoning is not reversible (McKinley *et al.*, 2002). Acetyl choline esterase inhibitors are also harmful to human when exposed (WHO 1973b).

2.1.8.2 Acetylcholine receptor stimulation

Some insecticides have the same effect as choline esterase inhibitors by acting as agonists of the acetylcholine receptor. Neonicotinoid insecticides act as acetylcholine analogue, that is, they mimic the action of the neurotransmitter by continually stimulating the nerve which leads to overstimulation of the nervous system and the insect dies. Spinosad is also an acetylcholine receptor agonist which has the same end result as Neonicotinoid (Rattan, 2010).

2.1.8.3 Chloride Channel Regulators

Chloride channel normally blocks reactions in some nerves and prevent excessive stimulation of the central nervous system (CNS). Gamma-Aminobutyric Acid (GABA) is an inhibitory neurotransmitter which, when activated at a synapse, the nerve impulse stops. Cyclodiene Organochlorine insecticides affect the chloride channel by inhibiting the GABA receptor. When a cyclodiene insecticide binds to the GABA molecule, the neurotransmitter can no longer close the chloride channel for which it acts as a gate. Thus there is continuous flow of electrical charge down the neuron, leading to overstimulation of the nervous system (Valles

and Koehler, 1997). Bifenazate (Hydrazine carboxylate class) is a GABA-gated chloride channel agonist which causes the gate to have the same action as GABA, closes the gate, making nerve impulses unable to travel down the chloride channel.

2.1.8.4 Sodium Channel Modulators

Pyrethrins and pyrethroids act on channels through which sodium is pumped to cause excitation of neurons. They prevent the sodium channels from closing, resulting in continual nerve impulse transmission, tremors, and eventual death of insects (Valles and Koehler, 1997).

2.1.8.5 Chitin Synthesis Inhibitors (CSIs)

Chitin is an important component of the insect's exoskeleton and moulting is necessary for the insect growth, to reach the adult stage and reproduce. Some insecticides block the synthesis of chitin, thus, the insects cannot moult to grow and reproduce which eventually die. Benzoylurea and cyromazine are examples of CSI (Alaa *et al.,* 1998).

2.1.8.6 Insect Growth Regulators (IGRs)

Hormones play various roles in moulting. Disruption of, or interference with any of these hormones inactivates the moulting process. IGRs, attack the insect's endocrine system, which produces the hormones needed for growth and development into an adult stage. Many IGRs mimic a special protein called juvenile hormone, which is circulated throughout the insect's body to maintain a current stage and the insects stop producing the juvenile hormone to metamorphose or change into next life stage. When an insect is poisoned by juvenile hormone mimics, the insect doesn't receive the signal to metamorphose because the IGR is still circulating throughout its body and sending the signal to stay in the current stage (El-Guindy *et al.,* 1982). Fenoxycarb, methoprene and hydroprene are examples of IGRs acting as juvenile hormone mimics. Ecdysone is another hormone important in metamorphosis.

Some insecticides such as Diacylhydrazine interfere with the production of ecdysone, making the insect unable to moult (Ascher *et al*., 1987). Azadirachtin, derived from neem oil, interferes with synthesis of insect development hormone, Prothoracictropic hormone (PTTH) and also acts as feeding deterrent (Senthilnathan *et al.,* 2008).

2.1.8.7 Energy Production Disruptors/Inhibitors

The main processes in energy production are electron transport and oxidative phosphorylation. Some insecticides inhibit or disrupt energy production. When electron transport is disrupted, oxidative phosphorylation is inhibited, and energy (ATP) cannot be stored for later use. Aliphatic organochlorides and amidinohydrazone interfere with complex I I of electron transport chain and shut down the target organism's ability to produce energy from its food. Some insecticides disrupt energy production through uncoupling oxidative phosphorylation from electron transport chain, thus no ATP is produced (Larson, 2001).

2.1.8.8 Mode of action of phytochemicals on target insect body

When insects feed on secondary metabolites they usually encounter toxic substances with relatively non-specific effects on a wide range of molecular targets. These targets range from proteins (enzymes, receptors, signalling molecules, ion-channels and structural proteins), hormones, nucleic acids, biomembranes, and other cellular components. This affects insect physiology in many ways and at various receptor sites, the principal of which is the nervous system such as, in neurotransmitter synthesis, storage, release, binding, and re-uptake, receptor activation and function, enzymes involved in signal transduction pathway (Rattan, 2010). The mechanism of action of plant secondary metabolites on insect body and physiological disruptions, such as inhibition of acetyl choline estrase (by essential oils), GABA-gated chloride channel (by thymol), sodium and potassium ion exchange disruption (by pyrethrin) and inhibition of cellular respiration (by rotenone) have been reported. Such

disruption also includes the blockage of calcium channels (by ryanodine), of nerve cell membrane action (by sabadilla), of octopamine receptors (thymol), hormonal balance disruption, mitotic poisioning (by azadirachtin), disruption of the molecular events of morphogenesis and alteration in the behaviour and memory of cholinergic system (by essential oil). Of these, the most important activity is the inhibition of acetylcholinerase activity (AChE) as it is a key enzyme responsible for terminating the nerve impulse transmission through synaptic pathway; AChE has been observed to be organophosphorus and carbamates resistant, and it is well-known that the alteration in AChE is one of the main resistance mechanisms in insect pests (Senthilnathan *et al.*, 2008).

2.1.9 Toxicity Response Determinant and Variation of plant derived larvicides

The efficacy of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant parts used, maturity of plant, solvents used for extraction as well as mosquito species. Variations were reported in the level of effectiveness of phytochemical compounds on target mosquito species in relation to; plant parts from which these active ingredients were extracted, responses in species and their developmental stages against the specified extract, solvent of extraction, geographical origin of the plant and photosensitivity of some of the compounds in the extract, (Sukumar *et al.*, 1991; Ghosh *et al*., 2012).

Changes were noticed in the larvicidal efficacy of the plant extracts due to geographical origin of the plant species as demonstrated in; *Citrus* sp, *Jatropha* sp, *Occimum sanctum*, *Momordica charantia*, *Piper* sp and *Azadirachta indica* (Karmegan, 1997; Rahuman *et al*., 2007; Rahuman and Venkatesan, 2008). While response variations in the different mosquito species studied was seen in *Curcuma domestica*, *Withania somnifera*, *Jatropha curcas*, *Piper retrofractum*, *Cestrum diurnum*, *Citrullus vulgaris*, and *Tridax procumbens* (Chansang *et al.*, 2005). Variation due to species of plant investigated was observed in *Euphorbia* sp,

Phyllanthus sp, *Curcuma* sp, *Solanum* sp, *Occimum* sp, *Eucalyptus* sp, *Plumbago* sp, *Vitex* sp, *Piper* sp, *Annona* sp and *Cleome* sp (Yadav *et al.*,2002; Krishnan *et al.*,2007; Rahuman and Venkatesan, 2008; Raghavendra *et al.*, 2009; Rawani *et al.*,2010) while response variation due to plant parts used to study the larvicidal efficacy was demonstrated in *Euphorbia tirucalli*, (Yadav *et al.*,2002) *Solanum xanthocarpum*, (Mohan *et al.*,2006) *Azadirechta indica*, (Haq *et al.*,1999; Mgbemena, 2010), *Solanum villosum*,(Chowdhury *et al.*,2008) *Annona squamosa*, (Kamaraj *et al*.,2010), *Withania somnifera* and *Occimum sanctum* (Anees, 2008; Kamaraj and Rahuman, 2010; Ghosh *et el.*, 2012). Response variation in different mosquito species was demonstrated in *An. stephensi*, *Ae. aegypti* and *Cx. Quinquefasciatus* with an LC_{50} values of 104.70, 138 and 83.18 ppm respectively, for 2nd instar larvae of three species after 24 hours of exposure (Kaushik and Saini, 2008), another study with fourth instar larvae of three species; *An. stephensi*, *Cx. quinquefasciatus*,and *Ae. aegypti* using *Eucalyptus citriodora* extract revealed an LC_{50} values 69.86, 81.12 and 91.76 ppm, respectively after 24 h and 26.7, 29.9 and 38.8 ppm, respectively after 72 h (Singh *et al.*,2007). Chansang *et al.*, (2005), reported LC_{50} values of 135ppm against *Cx. quinquefasciatus* and 79 ppm against *Ae. Aegypti* in study carried out with *Piper retrofractum* extract. *Solanum nigrum* LC_{50} values against fourth instar larvae of *An. Culicifacies*, *An. stephensi*, *Cx. quinquefasciatus*, *Ae. aegypti* were 9.04, 6.25, 12.25 and 17.63 ppm respectively (Raghavendra *et al.*, 2009). Protein isolated from *Solanum villosum* was tested against *An. stephensi*, *Cx.quinquefasciatus* and *Ae. aegypti* which showed LC_{50} values of 644.75, 645.75 and 747.22 ppm respectively (Chowdhury *et al.*, 2008). Larvicidal potency of the same plant was also demonstrated to vary with solvent (used for extraction) as shown in *Solanum nigrum* (n-hexane-9.04ppm; ethyl acetate-17.04ppm; aqueous-208.5ppm); *Momordica charantia* (n-hexane-1.2%, methanol-465.8ppm); *Azadirachta indica* (n-hexane-62.9ppm, methanol-824ppm, ethanol-390ppm); *Solanum xanthocarpum*(methanol-248.5ppm,

pet ether-41.28, CCl4-64.9ppm); *Anona squamosa* (methanol-2.26ppm, ethyl acetate-28.18ppm) and *Euphorbia tirucalli* (methanol-177ppm, pet ether-5.5ppm,Chloroform-200.7ppm) (Haq *et al.*,1999; Yadav *et al.*, 2002; Prabakar and Jebanesan, 2004; Mohan *et al.*, 2006; Raghavendra *et al.*, 2009; Rawani *et al.*,2009; Kamaraj *et al.*, 2010; Mgbemena, 2010). Potency variations have been attributed to change in active biochemical which depend upon the polarity of the solvents, ranging from most polar (e.g. water, methanol), moderately polar (e.g. ethyl acetate) to non-polar (e.g. pet ether and n-hexane) Ghosh *et al*., 2012.

2.1.10 Scope for isolation of toxic larvicidal active ingredients from plants

The efficacy of plant extracts as the reservoir pool of bioactive toxic agents against mosquito larvae have been documented in several studies. However, poor characterization and inefficiency to determine the structure of active toxic ingredients responsible for larvicidal activity have been identified as the reasons for the failure in laboratory to field utilisation of phytochemicals for commercialisation. Consequently, for the production of a green bio pesticide, the following steps have been recommended for research design with phytochemicals:

(i) Screening of floral biodiversity in search of crude plant extracts having mosquito larvicidal potentiality;

(ii) Preparation of plant solvent extracts starting from non-polar to polar chemicals and determination of the most effective solvent extract;

(iii) Phytochemical analysis of the solid residue and application of column chromatography and thin layer chromatography to purify and isolate toxic phytochemical with larvicidal activity;

(iv) Determination of the structure of active ingredients by infra-red (IR) spectroscopy, nuclear magnetic resonance (NMR) and gas chromatography and mass spectroscopy (GCMS) analysis;

(v) Study of the effect of active ingredients on non-target organisms e.g. Chironominae larvae; and

(vi) Field evaluation of the active ingredients for possible recommendation in vector control programme and commercial production (Ghosh *et al.*, 2012).

2.2 *Chromolaena odorata*

Chromolaena odorata (L.), R.M. King (*C. odorata*) formerly called *Eupatorium odoratum* L. is an aromatic and herbaceous perennial species which forms dense tangled bushes of about 1.5 - 2.0 meters in height. It is a member of the sunflower family, *Asteraceae*. The weed goes by many common names including Siam weed, devil weed, French weed, communist weed etc. (Vaisakh and Pandey, 2012). In Nigeria, the plant is referred to as '*obu inenawa'* or "*Obealiofulu*" by the Igbos and '*ewe awolowo'* by the Yorubas. *Chromolaena* is an important weed in tropical and subtropical areas extending from west, central and southern Africa to India, Sri Lanka, Bangladesh, Laos, Cambodia, Thailand, southern China, Taiwan and Indonesia.

Figure 2.3: *Chromolaena odorata* plant

Source: Cruttwell-McFadyen, 1991.

2.2.1 Classification of *C. odorata*

Kingdom*: Plantae*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Asterales*

Family: *Asteraceae*

Genus: *Chromolaena*

Species*: C. odorata*

2.2.2 Origin and Distribution

C. *odorata* is a native to the Caribbean and Latin America. In its native habitat, C. *odorata* occurs naturally over a wide range from southern Florida to northern Argentina, where it is generally not considered an important weed. C. *odorata* was first introduced into West Africa in the late 1930s, and is now estimated to be distributed from 12"W, 23'E, to 1O'N - 1O·S; this area extends from eastern Guinea to central Zaire and Central African Republic, southwards to northern Angola. C. *odorata* has also spread to the Natal coastal region of South Africa and has become a dominant weed in the humid and sub-humid zone of West and Central Africa. In Nigeria, C. *odorata* was first introduced around 1940 (Bennett, and Rao, 1968; Anon, 1983).

2.2.3 Traditional Uses of C. *odorata*

Many developing countries used this plant as a traditional remedy for variety of ailments. Plants decoction is taken as a remedy for cough and cold or in bath to treat skin diseases and it is a popular and effective therapy against diarrhoea, malaria, fever, tooth ache, diabetes, dysentery and colitis (Ajao *et al.*, 2011). The aqueous leaf extract of the plant is used as antiseptics for wound dressing and fresh juice from the leaf is also used as haemostatic to

arrest bleeding from fresh cuts and to stop nose bleeding (Phan *et al.*, 2001). In Southern Indian tradition, the leaf is ground into a paste and applied topically on affected places to heal wounds (Ayyanar and Ignacimuthu, 2009). These tradomedicinal values of plants have been claimed to lie in their phytochemical components (Anyasor *et al.,* 2011).

2.2.4 Phytochemical Composition of C. *odorata*

The phytochemical constituent of this plant was investigated by various scholars. Tests for tannins, steroids, terpenoids, flavonoids and cardiac glycosides were positive in methanolic and aqueous extracts. Alkaloids were detected only in the methanolic extract (Afolabi *et al*., 2007). The proximate and mineral content of *Chromolaena odorata* investigation showed a high total carbohydrate (20.58% W/W), crude fibre (10.76% W/W), and protein (6.56% W/W) with high calcium, iron, and sodium present. The anti-nutrients composition includes cyanogenic glycosides (0.05% W/W), phytates (0.22% W/W), saponins (0.80% W/W) and tannins (0.15% W/W) (Ngozi *et al.,* 2009). The essential oil components revealed the presence of α-pinene (42.2%), β-pinene (10.6%), germacrene D (9.7%), β-copaen-4α-ol (9.4%), (*E*)-caryophyllene (5.4%), and geijerene/pregeijerene (7.5%), (Moses *et al.,* 2010) .

2.2.5 Medicinal Values of C. *odorata*

The basis for the external applications and wound healing of *Chromolaena* was found to be its profound antioxidant action which helps in conserving the fibroblast and keratinocyte proliferation on the site of the wound (Phan *et al*., 2001). The phenolic acids present (protocatechuic, *p*-hydroxybenzoic, *p*-coumaric, ferulic and vanillic acids) and complex mixtures of lipophilic flavonoid aglycones (flavanones, flavonols, flavones and chalcones) were major and powerful antioxidants (Panda *et al.,* 2010). The extract also produced consistent analgesic, anti-inflammatory and antipyretic activities (Victor *et al*, 2005). Other studies have shown that the anti-inflammatory activity is accounted for, by the presence of

flavonoids in the extract (Ogunbiyi *et al.*, 2008).The *Chromolaena* extract was found to be cytoprotective in combination with honey when used in stomach ulcer lesions and this experiment in rats proved their efficacy as an antiulcer agent when used orally (Nur Jannah *et al.*, 2006). The aqueous extract of C. *odorata* was found to be bioactive as follows; (coagulation: 15.18 ± 0.023 min; clotting 0.26 ± 0.014 min); haemostatic activity than the ethanolic extract (Coagulation: 21.0 ± 0.696 min; Clotting 2.03 ± 0.035 min). These studies indicate that leaf extracts of *C. odorata* possess antioxidant, antibacterial and haemostatic activities.

2.2.6 Antibacterial effect of *C. odorata*

The antibacterial studies of *C. odorata* leave extracts show activity against *Klebsiella oxytoca*, *Salmonella enterica*, *Shigella sonnei* and *Vibrio cholera* (Atindehou *et al.*, 2013). Other studies showed that the ethanolic extract of C. *odorata* inhibited the growth of *S. aureus*, *S. typhi* and *E. coli* at various degrees while *S. aureus* and *S. typhi* only were inhibited by the aqueous extracts (Sukanya *et al.*, 2009; Lavanya and Brahmaprakash, 2011).

2.2.7 Toxicity of C. *odorata*

Cytotoxicity of the C*hromolaena* extract studied have demonstrated the presence of some compounds in the extract such as acacetin (5, 7- dihydroxy- 4'- methoxy flavone) and luteolin (5, 7, 3', 4'- tetrahydroxyflavone) which expressed cytotoxic activity against human small cell lung cancer and human breast cancer. The extract could be classified as being slightly toxic, since the LD_{50} was found to lie between 5.0g and 15.0g/kg body weight. This makes the preparation relatively safe for human use (Ogbonnia *et al.*, 2010).

2.3 *Persea americana*

Persea americana M., is an ever green flowering plant, belonging to the family, *Lauraceae*. The fruit of *Persea americana* is commonly known as Avocado pear or Alligator Pear, reflecting its shape and the leather like appearance of its peel. It is a plant from Central America (Mexico, Guatemala, Antilles), but it has shown easy adaptation to other tropical regions. Avocados are of dozens of varieties which fall into two categories; Mexico *Guatemalan* and West Indian species which differ in their size, appearance, and quality.

2.3.1 Classification

Kingdom: *Plantae*,

Division: *gnoliophyta*,

Class: *magnoliopsida*,

Order: *laurels*,

Family: *Lauraceae*,

Genus: *Persea*,

Species: *americana*

Binomial: *Persea americana M*.

Figure 2.4a: *Persea americana* Plant

Figure 2.4b: *P.america Fruit*

(**Source:** Haas, 1952)

2.3.2 Biological activities of *Persea americana* constituents

The fruit of *Persea americana* is eaten in many parts of the world. In recent years, research has focused on various parts of the plants. The fruit in particular has been shown to possess medicinal properties. The edible fruit pulp contains up to 33% oil rich in monounsaturated fatty acids (Ortiz *et al.,* 2004) that are believed to modify the fatty acid contents in cardiac and renal membranes and enhance the absorption of α/β-carotene and lutein (Salazar *et al*., 2005). The carotenoid content has been reported to play significant role in cancer risk reduction (Lu *et al.,* 2005). Other properties of the oil include wound healing (Nayak *et al.,* 2008) and hepatoprotection (Kawagishi *et al.,* 2001). Other parts of the plant have been reported to possess medicinal properties. The aqueous leaf extract for example has analgesic and anti-inflammatory (Adeyemi *et al.,* 2002), anticonvulsant (Ojewole and Amabeoku, 2006), hypoglycaemic and hypocholesterolaemic (Brai *et al.,* 2007), vasorelaxant and blood pressure reducing activities in animal studies (Owolabi *et al.,* 2005; Ojewole *et al*., 2007).

2.3.3 Phytochemical composition of *Persea americana* seed

According to Haas 1951, Avocado seed are usually considered as waste or as a source of seedlings suitable for use as rootstocks. Avocado seed contain relatively low amounts of calcium and magnesium, somewhat higher amounts of phosphorus, and high amounts of potassium.

The chemical composition of *P. americana* leaf, fruit and seed was investigated. The results obtained showed that the investigated samples contain phytochemicals such as phenols, saponins, and flavonoids in appreciable quantities. Proximate composition revealed that the fruit of *P.americana* contains more of fat and energy; seed, more of fat, protein and energy and the leaf, more of protein, fibre, and ash. Mineral contents of the investigated samples followed the order of leaf>fruit>seed in terms of concentration. These chemical compositions

of the investigated samples may be behind their medicinal values in phytomedicine (Arukwe *et al.,* 2012).

Phytochemical studies on avocado seeds have identified various classes of natural compounds such as phytosterols, triterpenes, fatty acids, furanoic acids, abscisic acid, proanthocyanidins, and polyphenols (Ding *et al.,* 2007; Leite *et al.,* 2009*).* In another study, Wang *et al.,* 2010, have reported the presence of catechin, epicatechin, and A- and B-type procyanidin dimers and trimers, tetramers, pentamers, and hexamers in the seed.

2.3.4 Tradomedicinal Uses of *Persea americana* Seed

Ethno pharmacological studies of the many cultures have reported the use of decoctions of avocado seeds for the treatment of mycotic and parasitic infections (Kunow, 2003). Seeds have also been reported for use against diabetes, inflammation, and gastrointestinal irregularity (Kunow, 2003; Ramos-Jerz, 2007).

Several beneficial medicinal properties of compounds present in the avocado seed and peel have been reported, which are related to the elevated levels of phenolic compounds (64% in seed, 23% in peel, and 13% in pulp). In addition, the seeds and peels of avocado also contribute 57% and 38% of the antioxidant capacities of the entire fruit, respectively. Avocado seeds have more antioxidant activity and polyphenol content than the pulp (Song and Barlow, 2004; Wang *et al*., 2010).

Pahua-Ramos *et al.,* 2012, investigated the effect of Avocado Seed Flour (ASF) on the lipid levels in mice on a hyperlipidemic diet and treatment with ASF significantly reduced the levels of total cholesterol and LDL-C. The antioxidant activity of phenolic compounds and dietary fiber in ASF could be responsible for the hypocholesterolemic activity of ASF in a hyperlipidemic model of mice. Antihypertensive, hypoglycaemic and cholesterol reducing tendency of *P. americana* seed was also demonstrated in rats (Imafidon *et al*., 2009).

2.3.5 Larvicidal and antimicrobial activities

The hexane and methanol extracts of avocado seeds were investigated for larvicidal effects against *Artemia salina* and *Aedes aegypti*. Antifungal potential against strains of *Candida* spp, *Cryptococcus neoformans* and *Malassezia pachydermatis* were evaluated by Leite *et al.*, 2009. Toxicity tests on *Artemia salina* and *Aedes aegypti* larvae show that, hexane and methanol extracts from avocado seeds against *Artemia salina,* have LC_{50} values of 2.37 and 24.13mg mL-1 respectively. While *Aedes aegypti* larvae show LC_{50} of 8.87mg/mL for hexane extract and 16.7mg/mL for methanol extract from avocado seeds. The extracts tested were also active against yeast strains tested *in vitro* (Leite, *et al.*, 2009). Studies on the antimicrobial activities of avocado seed acetone extract reveal antibacterial effects on *S. aureus*, *B. subtillis*, *Aspergillus glaucus* and *Penicillium notarum*, but there was no effect on *E. coli* and *Pseudomonas fluorescens* (Idris *et al*, 2009). Some of the phytochemicals are related to antifungal activity and larvicidal effect (Leite *et al.*, 2009).

2.3.6 Toxicity of *Persea americana* seed

Acute and sub-acute toxicity studies in rats were assessed which showed that the LD_{50} could not be determined after a maximum dose of 10 g/kg body weight. It was also reported that sub-acute treatment with the extract neither affected whole body weight nor organ-to-body weight ratios but significantly increased the fluid intake. Haematological parameters and the levels of ALT, AST, albumin and creatinine were not significantly altered. However, the concentration of total proteins was significantly increased in the treated group (Ozolua *et al*, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Silica gel, for column chromatography (60-230 mesh), Silica coated TLC plates (60 F_{254} Merck), pipette delivering 100-1000µl, Disposable tips (100µl, 500µl), 1ml pipettes, droppers with rubber suction bulbs, wire loops, nylon netting cage 75x35x35cm, 100ml disposable bowls, measuring cylinder and a strainer.

3.1.1 Chemicals

All chemicals used were of analytical grade and were purchased from reputable companies Silica gel.

Chemicals	Sources
1. ethanol	Sigma Aldrich Company, U.S.A.
2. ethyl acetate	Sigma Aldrich Company,U.S.A.
3. n-hexane	Sigma Aldrich Company,U.S.A
4. Dimethyl sulfoxide (DMSO)	Qualichem Chemical company, Germany
5. Silica gel(60-230 mesh)	Qualichem Chemical company, Germany

3.1.2 Plant Collection and identification

Persea americana fruits were obtained in 2013, from Nnewi town, Anambra State, by Humphrey Nzelibe, while the *C. odorata* leaves were also obtained in 2013, from

undeveloped land, behind Public Service Institute of Nigeria, Dutsen Alhaji, suburb of Abuja-Nigeria. The plants were identified and authenticated in Herbarium of the Department of Biological Sciences, Ahmadu Bello University Zaria.

3.2 Methods

3.2.1 Preparation of extracts

The seeds of *Persea americana* were extracted from the ripened fruits and chopped into pieces for shade drying while the leaves of *C. odorata* obtained were shade dried at room temperature. Each of the dried plant material was ground into powder using laboratory milling machine in the Food Science and Technology Programme Dept. of the I.A.R., A.B.U. Zaria. The powdered plant materials were used to prepare the crude extracts using distilled water, ethanol, ethyl acetate and n-hexane. Each powdered plant material (100g) was weighed and put in separate beakers and 1000ml of each solvent (water, ethanol, ethyl acetate and n-hexane,) were added to each of the beakers with periodic shaking for 24 hours followed by filtration using Whatman No.1 filter paper. The filtrates were concentrated using rotary evaporator except for the aqueous which was allowed to concentrate in water bath set at 45°c. The extracts obtained were labelled and stored at room temperature in amber coloured air tight bottle.

3.2.2 Rearing of Mosquito Larvae

Aedes vittatus mosquito larvae were collected from Kufena Rock in Zaria. The larvae were identified and authenticated at the Entomology Research Laboratory of Department of Biological Sciences A.B.U. Zaria. The larvae were reared in plastic and enamel trays in tap water (boiled and cooled). They were maintained at room temperature and fed a diet of brewer's yeast and biscuits in a ratio of 1:1. Pupae were transferred from the trays to a cup containing tap water and placed in screened cages where adults emerged. Adults of *Aedes*

vittatus were reared in wooden cages. Adults were continuously provided with 10% sucrose solution soaked on a cotton pad, placed at the middle of the cage. They were provided with a Guinea pig placed in restrained position overnight for blood feeding. A petri dish with moisten filter paper was provided in the cage for oviposition and was maintained at the same environmental condition. The third and fourth instars larvae of the second generation were used for the Bioassay (Senthilnathan, 2008).

3.3 Phytochemical Analysis

Phytochemical analysis was carried out on the two plant extracts using a standard procedure for identification of phytochemical constituents as described by Harborne (1973), Trease and Evans, (1989) and Sofowora (1993).

3.3.1 Test for Saponins

About 2g of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. About 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsification.

3.3.2 Test for tannins

About 0.5g of the dried powdered sample was boiled in 20ml of water in a test tube then filtered and 1ml of 0.1% ferric chloride was added and observed for the brownish green or a blue-black colouration.

3.3.3 Test for flavonoids

Dilute ammonia solution (5ml) was added to a portion of aqueous filtrate followed by addition of concentrated sulfuric acid. A yellow colouration observed indicate the presence of flavonoids.

3.3.4 Test for sterols

About 2ml of acetic anhydride was added to 0.5g of Ethanolic extract of the sample with 2ml sulfuric acid. The colour change from violet to blue or green indicates the presence of sterols.

3.3.5 Test for Terpenoids

Using Salkowski's test, 5ml of the extract was mixed in 2ml of chloroform and 3ml of concentrated sulfuric acid was carefully added to form a layer. A reddish brown colouration of the interface formed will show a positive result for the presence of Terpenoids.

3.3.6 Test for Anthracenes

The test for Anthracenes of 5ml aqueous extract was shaken with equal volume of chloroform and allowed to form a chloroform layer. 10ml of ammonia was added, shaken vigorously and then allowed to separate

3.3.7 Test for cardiac glycosides

The test for cardiac glycosides was carried out using the Keller-Kiliani test. About 5ml of extract was treated with 2ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulfuric acid. A brown ring of interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear above and below the brown ring, formed just gradually throughout thin layer. This indicates the presence of cardiac glycosides.

3.3.8 Test for alkaloids

About 1 ml of the aqueous plant extract was treated with 2ml of picric acid solution. Formation of orange colouration indicates the presence of alkaloids.

3.4 Preparation of Stock Solutions

A Stock solution of 20ml of 1% was prepared by weighing 200mg of the crude extract in a small vial, to which is added, a drop of the solvent used, DMSO, and adding distil water to make up 20ml, as the stock solution. The solution was kept in a screw-cap vial, with aluminum foil over the mouth of the vial until required for immediate use WHO, 2005.

3.4.1 Preparation of Test Concentrations for Bioassay

The stock solution prepared was serially diluted according to WHO (2005) guidelines. Triplicate of test volumes were prepared into final concentration of 100ml. Six concentrations of aqueous, ethanolic, ethyl acetate and n-hexane extract at, 12.5ppm, 25ppm, 50ppn, 100ppm, 200ppm, 300ppm and 600ppm in previously boiled and cooled tap water (dechlorinated water).

3.5 Larvicidal Bioassay

Bioassay was performed according to WHO (2005) guidelines. After preparing test concentrations, fifteen 3r^{d} and 4th instar larvae were introduced into each plastic bowl (100 ml capacity). The mosquito larvae were exposed to a wide range of test concentrations and a corresponding control to find out the larval mortality. The number of dead larvae was recorded after six hours interval for 24 hours and the percentage mortality was calculated. Batches of 15 third and fourth instar larvae were transferred by means of strainers, screen loops or droppers to small disposable test cups, each containing 100ml of water. The depth of the water in the bowls were maintained between 5 cm and 10 cm to avoid undue mortality. The experiment was set up in triplicates for each concentration with an equal number of controls simultaneously with tap water, to which 1 ml of respective organic solvents was added. The test containers were maintained at room temperature with a photoperiod of 12hrs light followed by 12 hrs dark (12L: 12D).

3.5.1 Determination of Lethal Concentrations

The number of dead larvae at various concentrations recorded from the triplicates readings were used to generate a dose response plots. The lethal concentrations (LC_{50} and LC_{90}) were calculated using Probit analysis (Finney, 1971). The percentage mortality calculated and corrections for mortality where necessary were done by using Abbot's (1925) formula.

Moribund larvae were counted and added to dead larvae for calculating percentage mortality.

The mortalities of treated groups was corrected according to Abbott's formula (Abbott, 1925).

$$\text{Mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Number of larvae exposed}} \times 100$$

$$\textit{Corrected Mortality}\ (\%) = \frac{X - Y}{X} \times 100$$

Where X = percentage survival in the untreated control and

Y = percentage survival in the treated sample.

3.6 Thin Layer Chromatography (TLC)

Thin Layer Chromatography was carried out to determine the best solvent system for column. A chromatographic Silica gel 60 F_{254} plate was used. The sample was dissolved in n-hexane and then applied to the plate using a micro haematocrit capillary tube until the quantity loaded was adjudged sufficient for the experiment. The plate was placed in a chromatographic tank and developed with a mixture of hexane/Ethylacetate (9:1 to 1:9) ratio.

The plate was removed from the TLC tank, air dried and visualised with 20% sulpuric acid in alcohol under UV light (Ejele *et al*., 2012).

3.6.1 Column Chromatography

The chromatographic column was packed with (80g) silica gel 60-120 (the stationary phase). The crude n-hexane extracts (4g) of *Persea americana* seed and *C. odorata* leaves were separately mixed with silica gel and loaded on to the column. The components of the crude n-hexane extracts were separated by gradient elution on a silica packed column using an eluting mixture of n-hexane : ethyl acetate (starting from 100% n-hexane; n-hexane 9.8 : Ethyl acetate 0.8, 9.5:0.5, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6 and then 100% ethyl acetate) obtained from TLC. Fractions of 30ml aliquots were collected into different beakers. The solvent was allowed to evaporate and the contents were profiled on TLC plate (Ejele *et al*., 2012). Fractions with similar TLC profiles were pooled together. Larvicidal activities of the fractions were tested against *Aedes vittatus* mosquito larvae. The fractions with the highest larvicidal activity were selected for spectroscopic analysis.

3.7 Characterization of Larvicidal Compounds in the Bioactive Fraction by GC/MS and FTIR Spectroscopy

3.7.1 Fourier Transform Infra-Red Spectroscopy

The FTIR analysis of the most active fraction, with the highest larvicidal property was carried out to determine functional group(s) present (Shidmazu model), available at the National Research Institute for Chemical Technology (NARICT) Laboratory Zaria.

3.7.2 Gas Chromatography/Mass Spectroscopy

For the identification of fractions showing highest larvicidal activity, the samples were subjected to GC-MS analysis. The samples (1 µl) was injected into a RTX-5 column (60 mX

0.25 mm i.d., film thickness 0.25 µm) of GC-MS (model GC-MS-QP-2010 plus, Shimadzu, Japan). Helium was used as carrier gas at a constant column flow rate of 1.58 ml/min at 108 kpa inlet pressure. Temperature programming was maintained from 100°C to 200 °C with constant rise of 5 °C/min and then held isothermal at 200 °C for 6 min; further the temperature was increased by 10 °C/min up to 290 °C and again held isothermal at 290 °C for 10 min. The injector and ion source temperatures were 270 °C and 250 °C, respectively. The most active fractions NHP6 and NHC6 (2 mg/ml) were dissolved in ethyl acetate and n-hexane (HPLC grade, Merck, India) before injection. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 800 Dalton. The final confirmation of constituents was made by computer matching of the mass spectra of peaks with the National Institute Science and Technology (NIST) libraries 2005 mass spectral database at the National Research Institute for chemical Technology (NARICT), Zaria Kaduna State, Nigeria.

3.8 Statistical Analysis

Data from mortality response of solvent extracts used and fractions investigated was expressed as Percentage Mortality and Lethal concentration (LC_{50} and LC_{90}) by Log Probit Analysis using IBM SPSS Version 20.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Constituents of Extracts of *Persea americana* Seed and *Chromolaena odorata* Leaf

The screening was carried out, to determine the phytochemical constituents of the different solvent extracts. Thus, test for presence of alkaloids, saponins, flavonoids, terpenoids, steroids, tannins and cardiac glycosides was performed on each of the crude extracts, the result of which is presented in Table 4.2 below. Alkaloids were not detected in both plant extracts. Saponins were present only in ethanolic and ethyl acetate of *P. americana* seed extracts but absent in *C. odorata* extracts. Flavonoid was found in ethanolic and ethyl acetate of *P. americana* but absent in n-hexane extracts of this plant. Flavonoid was found to be present in ethanolic extract of *C. odorata* only, but absent in ethyl acetate and n-hexane extracts of this plant. Steroids, cardiac glycosides and terpenoids were all detected in the various solvents extracts of the two plants. Tannins were not detected in all the solvent extracts of the two plants.

Table 4.1: Qualitative Phytochemical Constituents of *P. americana* and *C. odorata* Extracts from Different Solvents.

pyhtochemical	*P.americana* seed			*C. odorata* leaf		
	Ethanol	ethylacatate	n-hexane	Ethanol	ethylacatate	n-hexane
Alkaloid	-	-	-	-	-	-
Saponins	+	+	-	-	-	-
Flavonoids	+	+	-	+	-	-
Sterols	+	+	+	+	+	+
Tannins	-	-	-	-	-	-
Cardiac Glycosides	+	+	+	+	+	+
terpenoids	+	+	+	+	+	+

Key: present (+), absent (-)

4.2 Larvicidal Activity of *Persea americana* Seed Extracts against *Aaedes vittatus*

The larvicidal bioassay carried out using different solvents (ethanol, ethyl acetate and n-hexane) extracts of the plant materials gives the result shown in the Table 4.2 below. The solvents were chosen based on polarity starting with polar solvent, ethanol; moderately polar solvent, ethyl acetate; and non-polar solvent, n-hexane. Thus, table 4.2 shows the larvicidal activity of extracts of *P. americana* seed against *Aedes vittatus* at different time interval post exposure. The mortality was expressed in percentage after which probit analysis of the observed responses against concentration was statistically ran using SPSS to establish the LC_{50} and LC_{90} which gives the potency index of the various solvent extracts tested.

From the table 4.2, the percentage mortality of the plant was highest in n-hexane extract of *P.americana* with 100% right from 50ppm after 12 hours of exposure and 100ppm after 6 hours of exposure. In the other two solvents, 100% mortality was recorded at 200ppm after 12hours of exposure in ethanolic extract whereas same record was only obtained at 600ppm after 12hours of exposure in the case of ethyl acetate extracts of the plant. Thus, it can be observed that n-hexane extract of *P. americana* shows 100% mortality at 100ppm and 50ppm after 6 hours and 12 hours of exposure respectively, with LC_{50} of 0.827ppm while the ethyl acetate extract shows 100% mortality at 600ppm after 12 hours of exposure, with LC_{50} of 2.799ppm.

Table 4.2: Percentage Mortality and Lethal Concentration of *P. americana* Seed Extracts against Aedes vittatus Larvae.

Solvents Extract	Mortality (%)							Lethal Conc.(ppm)	
	Conc./ Exposure Time(hr)	25ppm	50ppm	100ppm	200ppm	300ppm	600 ppm	LC_{50}	LC_{90}
	6	7	20±7.0	20	33	27±7.00	40		
Ethanol	12	40±7.0	86±7.0	73±10.0	100	100	100	1.799	10.242
	24	40±0.0	86±4.0	73	100	100	100		
	6	0	0	20±7.0	20	33±7.0	40		
Ethylacetate	12	27	13±7.0	87	87±10.3	93	100	2.732	9.016
	24	33±7.0	40	93	93±7.0	93	100		
	6	40±7.0	40	100	100	100	100		
n-hexane	12	60±7.0	100	100	100	100	100	0.827	1.972
	24	100	100	100	100	100	100		

Values represent Mean ± SD (n=15 and r=3)

4.3 Larvicidal activity of *Chromolaena odorata* leaf extracts against *Aedes vittatus* Larvae

Table 4.3, shows larvicidal activity of extracts of *C. odorata* leaves at different concentrations, after 6, 12, and 24 hours of exposure. From the table 4.3, the mortality percentage was observed, with n-hexane extracts having 100% mortality at 100ppm after 12hour of exposure, whereas the same results were recorded in ethanol extract at 300ppm after 24 hour of exposure and 600ppm in case of ethyl acetate. Furthermore, it can be observed that n-hexane extracts of *c .odorata* with LC_{50} of 1.833ppm is most potent compared to ethanol and ethyl acetate extracts with LC_{50} of 3.314ppm and 5.163ppm respectively.

Table 4.3: **Percentage Mortality and Lethal Concentrations of *C. odorata* Leaf Extracts against *Aedes vittatus* Larvae.**

Extraction Solvent	Mortality (%)							Lethal Conc. (ppm)	
	Conc./ Exposure Time(Hr)	25ppm	50ppm	100ppm	200ppm	300ppm	600 ppm	LC_{50}	LC_{90}
Ethanol	6	0	0	13	20±7.0	33	40±7.0		
	12	0	0	73±10.3	73	80±7.0	93±7.0	3.314	7.772
	24	13	33±7.0	93±7.0	93	100	100		
Ethylacetate	6	0	0	0	13±7	13	27		
	12	7	13±7.0	13	27±7	47±7.0	60	5.163	26.222
	24	33	53±7.0	60±7.0	80	87±7.0	100		
n-hexane	6	0	7±2.0	20±2.0	27±2.3	40±6.0	40		
	12	13±2.0	80±2.0	100	100	100	100	1.835	7.995
	24	60±5.0	86±5.0	100	100	100	100		

Values represent Mean ± SD (n=15, r=3)

4.4 Larvicidal Activity of Chromatographic Fractions of n-hexane Extracts (CFnHE) of *Persea americana* Seed against *Aedes vittatus* Larvae

Table 4.4 shows the larvicidal activity of the chromatographic fractions of *P. americana* n-hexane extracts against the *Aedes vittatus*. Seven fractions were obtained. From Table 4.4, increased potency of some fractions (fraction 6, nHP6 in particular) was noticed at 25ppm. The highest mortality of 100% was recorded at 25ppm after 12hours of exposure in fraction 7(nHP7) of *P.americana* followed by 100% mortality at 100ppm after 12 hours of exposure in fraction 6 (nHP6); fractions 4 and 5 show 100% mortality at 100ppm and 200ppm after 12 hours and 24 hours of exposure respectively, while fractions nHP3, nHP2, and nHP1 show percentage mortality of 98, 91 and 64 at 300ppm after 24 hours of exposure. The LC_{50} of the various CFnHE was found, which show 0.486ppm for nHP6 as most toxic fraction, which is followed by nHP7 and nHP4, having LC_{50} values of 0.727ppm, 1.509ppm respectively. These show higher potency compared to the n-hexane crude extracts of the plant. Whereas Fractions; nHP1, nHP2 and nHP3, show lower toxicity with LC_{50} of 8.024ppm, 4.674ppm and 4.622ppm respectively.

Table 4.4: Percentage Mortality and Lethal Concentrations of Chromatographic Fractions of *P. americana* Seed against *Aedes vittatus* Larvae.

Fractions (CFnHE)	Mortality (%)							Lethal Conc. (ppm)	
	Conc./ Exposure Time(Hr)	12.5ppm	25ppm	50ppm	100ppm	200ppm	300ppm	LC_{50}	LC_{90}
nHPa1	6	2±1.0	0	2±2.0	7±2.0	20±2.1	31±1.2		
	12	4±1.0	9±3.0	16±4.0	29±4.0	40±7.0	33±2.0	8.024	32.076
	24	11±3.1	11±3.1	22±2.0	36±4.0	51±3.0	64±3.1		
nHPa2	6	0	2±1.1	13	24±4.0	33±4.0	36±2.1		
	12	0	16±4.0	18±1.2	42±4.0	62±4.0	82±5.0	4.674	9.993
	24	4±4.0	18±3.0	22±2.0	49±3.1	71±1.0	91±2.0		
nHPa3	6	0	18±1.0	4±4.0	9±2.0	18±5.0	29±7.0		
	12	4±3.0	31±2.0	38±3.0	29±3.0	64±2.0	78±3.1	4.622	21.867
	24	27±4.0	40±7.0	56±4.0	60	89±3.0	98±2.0		
nHPa4	6	20±3.0	29±3.1	40±7.0	47±3.5	47	53±3.0		
	12	36±3.0	51±5.1	78±9.0	91±5.0	100	100	1.509	8.337
	24	71±6.0	78±5.0	98±2.0	100	100	100		
nHPa5	6	0	0	7±4.0	13±7.0	20	47±10		
	12	0	20±4.0	27±6.0	40±4.0	33±9.0	100	3.679	18.577
	24	60	63±3.0	80±3.0	100	87±2.0	100		
nHPa6	6	24±4.0	36±3.0	58±4.0	56±10.0	64±4.0	84±8.0		
	12	84±3.0	93±7.0	96±4.0	100	100	100	0.486	5.130
	24	91±3.0	93±3.0	100	100	100	100		
nHPa7	6	9±6.0	7	7	38±5.0	56±2.0	82±10		
	12	89±7.0	100	100	100	100	100	0.727	8.875
	24	91±2.0	100	100	100	100	100		

Values represent Mean ± SD (n=15, r=3), *P. americana-Pa*

4.5 Larvicidal Activity of Chromatographic Fractions of n-hexane Extract (CFnHE) of *Chromolaena odorata* Leaf against *Aedes vittatus* Larvae

Table 4.5 below, shows the percentage mortality and lethal concentrations of chromatographic fractions of n-Hexane extract (CFnHE) of *C. odorata* leaf after 6, 12 and 24 hour of exposure. It can be observed, from the Table 4.5, eleven fractions were tested against the *Aedes vittatus* larvae. The mortality of 100% at 100ppm was observed in fractions; nHCo3 (6 hours post exposure), nHCo1 (12 hours post exposure) and nHCo5. CFnHE, such as nHCo6 and nHCo4 show 100% mortality at 200ppm after 12 hours and 24 hours of exposure respectively. There was no mortality observed in fraction 7-nHCo7 at all concentrations (tested) throughout the exposure period of 24 hours. Fraction 9-nHCo9 shows 100% mortality at 300ppm after 24 hours of exposure while in fraction 8-nHCo8, the highest mortality (87%) observed was at 300ppm, preceded by fraction 10-nHCo10 (80%) and fraction 11-nHCo11 (67%).

The toxicity index (LC_{50}) of the fractions show that, nHCo6 and nHC03 are most toxic, having LC_{50} of 1.308ppm and 1.668ppm respectively. These were followed by nHCo5, nHCo1 and nHCo3 with LC_{50} values of 1.811ppm, 2.343ppm and 2.721ppm respectively. Fractions nHCo4, nHCo10, nHCo9 and nHCo8 were found to have LC_{50} values of 3.263ppm, 4.48ppm, 5.05ppm and 5.543ppm respectively, while nHCo11 was found to be least toxic, having LC_{50} of 16.74ppm. The nHCo7 is non-toxic to the larvae within the range of these test concentrations and exposure period.

Table 4.5: Percentage Mortality and Lethal Concentrations of Chromatographic Fractions of *Chromolaena odorata* Leaf against *Aedes vittatus* Larvae.

Fractions CFnHE	Mortality (%)							Lethal Conc.(ppm)	
	Conc./ Exposure Time(Hr)	12.5ppm	25ppm	50ppm	100ppm	200ppm	300ppm	LC_{50}	LC_{90}
nHCo1	6	0	0	15±6.0	66±10.0	42±6.0	62±3.0		
	12	0	0	87±7.0	100	100	100	2.343	5.470
	24	0	0	95±5.0	100	100	100		
nHCo2	6	0	9±2.0	11±3.0	27±8.0	29±4.0	36±4.0		
	12	7	62±5.0	69±4.0	78±5.0	87±4.0	91±4.1	2.721	9.313
	24	13±7	87	91±3.1	93	98±2.0	100		
nHCo3	6	11±3.1	36±10.0	49±9.1	100	100	100		
	12	24±4.0	52±7.0	64±3.1	100	100	100	1.668	3.636
	24	47	71±4.0	87±6.0	100	100	100		
nHCo4	6	0	7±4.0	4±3.0	18±10.0	47±7.0	60		
	12	11±4.0	18±6.1	40±6.1	71±3.1	76±4.0	80±7.0	3.263	8.953
	24	22±10.0	42±7.1	76±4.0	93	100	100		
nHCo5	6	2±2.0	9±5.0	24±4.0	44±6.1	73	93		
	12	29±3.0	58±4.0	64±4.0	100	100	100	1.811	5.299
	24	67±7.0	82±10.0	91±3.1	100	100	100		
nHCo6	6	9±3.1	16±4.0	33±7.0	47	60±7.0	100		
	12	56±4.0	73±7.0	84±4.0	87±7.0	100	100	1.308	5.576
	24	80	93	84±10	100	100	100		
nHCo7	6	0	0	0	0	0	0		
	12	0	0	0	0	0	0	0	0
	24	0	0	0	0	0	0		
nHCo8	6	0	7±7.0	13±7.0	20	31±3.1	0		
	12	13±7.0	18±5.0	26±7.0	51±3.1	6210.0	67	5.543	44.421
	24	36	44±4.0	60±4.0	69±3.1	80±7.0	87		
nHCo9	6	0	0	0	0	9±3.1	13		
	12	0	11±4.0	18±4.0	29±10.0	60	73	5.058	12.740
	24	4±3.0	18±10.0	33±3.0	78±4.0	89±7.1	100		
nHCo10	6	0	0	0	9±3.1	18±7.0	33±10.1		
	12	0	0	18±7.1	64±4.0	67	80	4.48	9.953
	24	0	0	44±6.1	80	78±4.0	80		
nHCo11	6	7	31±3.0	22±7.1	0	20	13		
	12	16±4.0	42±4.0	42±4.0	24±10.0	33	47	16.74	2075
	24	29±3.0	51±3.1	53±7	42±4.0	53±7.0	67		

Values represent Mean ± SD (n=3, r=15)

4.6 GC/MS Characterisation of the Most Potent Fraction (nHPa6) of *P. americana*

The plate 4.1 below shows the GCMS spectra (abundance against retention time) of the most potent fraction nHPa6 of *P. americana* which revealed eighteen peaks, four of which are prominent. Table 4.6 shows the peaks with their corresponding percentage abundance, compound name, molecular structure and similarity index as compared with NIST library. The prominent peaks with abundance greater than 10%, include; 13, 3, 17 and 18, which correspond to Oleic Acid (22.23 %), 3-Hydroxy-2,2,4-trimethyl pentylester of isobutanoic acid (12.26%) Methyl 2-(acetyloxy)hexadecanoate (10.28%) and 3,4-Dimethyl-1-decene (10.02%) with similarity index (SI) of 92, 91,83 and 85 respectively.

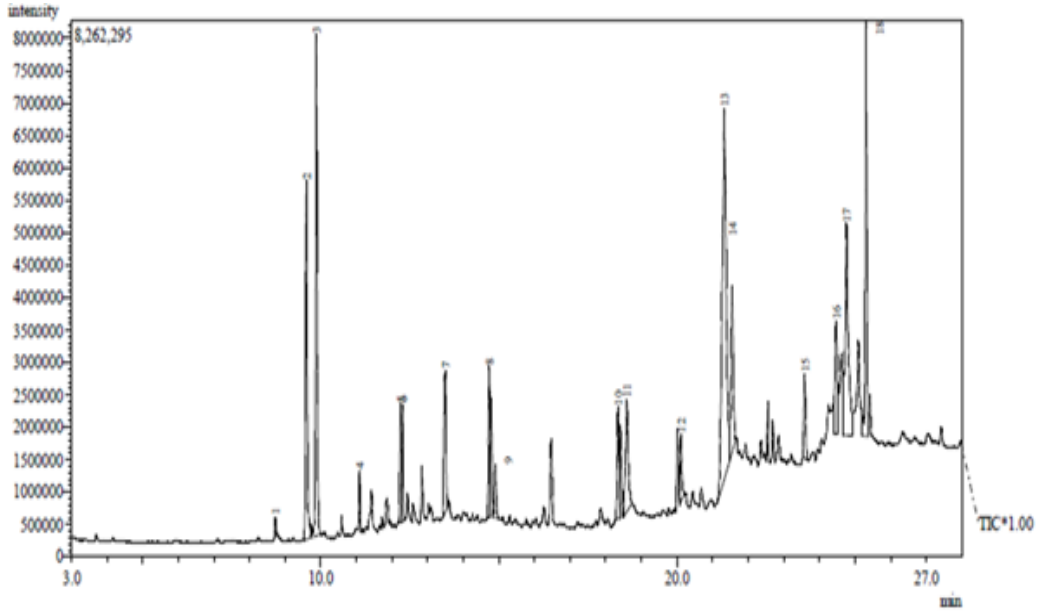
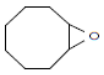
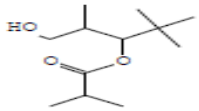
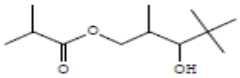
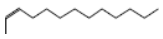
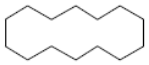
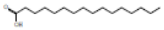
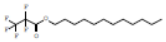
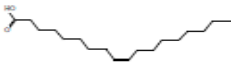
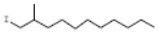
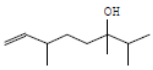
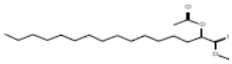
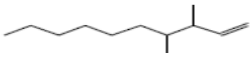


Plate 4.1: GC/MS SPECTRA of *P.americana* FRACTION SIX (nHPa6)

Table 4.6: GC/MS Spectral Interpretation of *P. americana* Most Potent Chromatographic Fraction (nHPa6)

Peak	RT (min)	Area (%)	Compound	SI	Structure	Molecular weight
1	8.72	0.41	1,2-Epoxycyclooctane	90		126
2	9.60	9.23	2,2-Dimethyl-1-(2-hydroxy-1-isopropyl)propyl ester of isobutanoic	93		216
3	9.88	12.26	3-Hydroxy-2,2,4-trimethyl pentylester of isobutanoic acid	91		216
4	11.09	1.02	n-Tridecane	95		184
5	12.24	2.02	(2Z)-2-Tridecene	96		182
6	12.30	1.89	Hexadecane	96		226
7	13.50	4.77	2,6-Dimethylheptadecane	97		268
8	14.73	3.35	n-Hexadec-1-ene	95		224
9	14.90	1.43	n-Dodecane	95		170
10	18.35	2.82	Cyclotetradecane	94		196
11	18.59	4.72	Hexadecanoic acid	93		226
12	20.12	1.81	Pentafluoropropionic acid, dodecyl ester	94		332
13	21.35	22.23	Oleic Acid	92		282
14	21.55	5.70	Nonadecanoic acid	89		298
15	23.59	2.07	1-Iodo-2-methylundecane	94		296
16	24.48	3.94	2,3,6-Trimethyl-7-octen-3-ol	87		170
17	24.77	10.28	Methyl 2-(acetyloxy)hexadecanoate	83		328
18	25.32	10.02	3,4-Dimethyl-1-decene	85		168

4.7 GC/MS Characterisation of the Most Potent Fraction (nHCo6) of *C. odorata*

The plate 4.2 below shows GCMS spectra (abundance against retention time) of the most potent fraction (nHCo6) of *C. odorata*, which revealed thirteen peaks, two of which are prominent and most abundant. Table 4.7 shows the peaks with their corresponding retention time, percentage abundance, and compound structure and similarity index (SI) compared with NIST library. It can be observed from table 4.7, that peaks ten and eleven are the prominent and most abundant which correspond to oleic acid and octadecanoic acid with 48.58% and 24.38% abundance and similarity index of 93 and 91% respectively. Other compounds detected with greater than 2% abundance include; hexadecanoic acid, octadecenyl aldehyde, 1-tetracosanol, 1-pentadecene and 2,6-Dimethylheptadecane.

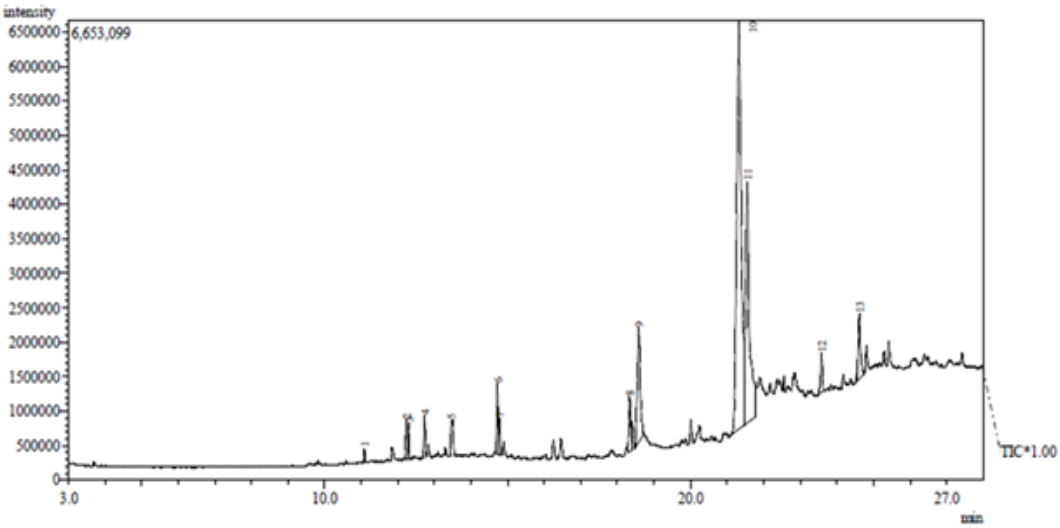
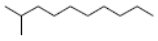
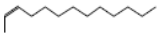
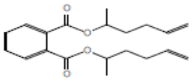
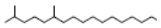
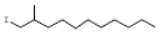
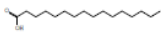
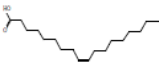
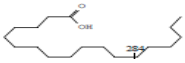
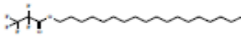


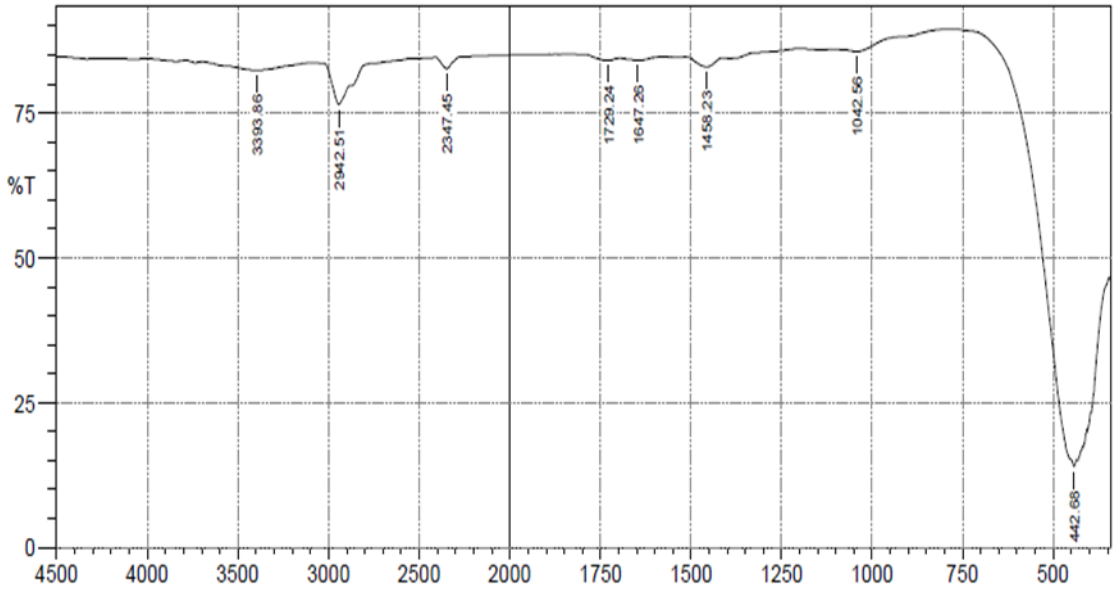
Plate 4.2: GC/MS Spectra of Most Potent Chromatographic Fraction Six (nHco6) of *C. Odorata*

Table 4.7: GCMS Spectral Interpretation of Most Potent Chromatographic Fraction Six (nHCo6) of *C.odorata*

Peak	RT (min)	Area(%)	Compound	SI	Structure	Molecular weight
1	11.09	0.40	Decane, 2-methyl	93		156
2	12.24	1.18	2-Tridecene	95		182
3	12.30	1.07	n-Tridecane	95		
4	12.73	1.64	Phthalic acid, di-(1-hexen-5-yl) ester	81		194
5	13.496	2.18	2,6-Dimethylheptadecane	95		268
6	14.73	2.61	1-Pentadecene	94		210
7	14.79	1.35	1-Iodo-2-methylundecane	95		296
8	18.34	2.54	1-Tetracosanol	95		354
9	18.59	8.84	n-Hexadecanoic acid	94		256
10	21.33	48.58	Oleic Acid	93		282
11	21.55	24.38	Octadecanoic acid	91		284
12	23.59	1.71	Pentafluoropropionic acid, octadecyl ester	91		416
13	24.00	3.47	Octadecenyl aldehyde	87		266

4.8 Functional Group Identification of nHPa6

FTIR analysis carried out on the most potent fraction, nHPa6, revealed various functional groups present. Plate 4.3 shows the spectra (% transmittance against wave number) which depicts eight different absorption bands at 442.68, 1042.56, 1458.23, 1647.26, 1727.24, 2347.45, 2942.51 and 3393.86 cm^{-1}(wave numbers). These absorption frequencies correspond to various functional groups as shown in Table 4.8, which include; alkyl halides (442.68cm^{-1}), C-O stretch for COOH ester and ether (1042.56 cm^{-1}), alkenes C-H stretch (1458.23cm^{-1}), alkenes C=C- (1647.26 cm^{-1}), esters C=O stretch (1727.24cm^{-1}), alkane (2942.51cm^{-1}), and carboxylic acids O-H stretch (3393.86 cm^{-1}).



1/cm

Plate 4.3: FTIR Spectra of *P.americana* Fraction Six (nHPa6)

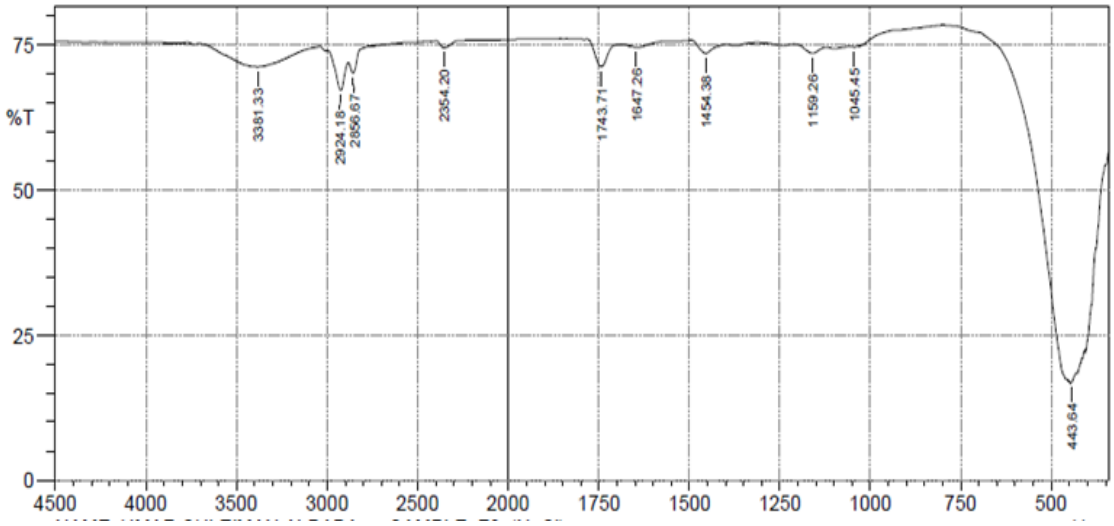
Table 4.8: Fourier Transformed Infrared Spectroscopy (FTIR) Analysis of *P. americana* Fraction Six (nHPa6)

Peak No.	Frequency (cm^{-1})	Intensity	Type of Bond	Functional Group	Vibration
1	442.68	15.29	C-Br stretch	Alkyl halides	M
2	1042.56	10.17	C-O stretch	Alcohol, COOH Ester	S
3	1458.23	7.17	C-H stretch	Alkenes	M
4	1647.26	0.87	-C=C- stretch	alkenes	M
5	1727.24	0.29	C=O stretch	Carbonyl ester, Sat Aliphatics	S
6	2347.45	11.41	C≡N stretch	Nitrile	M
7	2942.51	14.76	C-H	Alkanes	M
8	3393.86	1.87	O-H stretch	Carboxylic O-H	M

M-medium, S- strong vibrational mode

4.9 Functional Group Identification of nHCo6

The FTIR spectra, plate 4.4, for the most potent fraction, nHCo6, of *C. odorata* shows ten absorption bands at different frequencies which characterised various functional groups present. Table 4.9 shows the peaks with their corresponding frequencies, type of bond and functional groups detected. These include; alkyl halide C-Br stretch (443.64cm^{-1}), esters C-O stretch (1045.45 cm^{-1}), alkyl halide C-H wag (1159.26 cm^{-1}), alkanes C-H bend (1454.38 cm^{-1}), alkenes -C=C- stretch (1647.26 cm^{-1}), esters C=O stretch (1743.71 cm^{-1}), alkanes C-H stretch (2856.57 cm^{-1}) and alcohol O-H stretch (3381.33 cm^{-1}).



1/cm

Plate 4.4: FTIR Spectra of *C. odorata* Fraction nHCo6

Table 4. 9: Fourier Transformed Infrared Spectroscopy (FTIR) Analysis of *C. odorata* Fraction Six (nHCo6)

PEAK NO	FREQUENCY (CM^{-1})	INTENSITY AREA	TYPE OF BOND	TYPE OF FUNCTIONAL GROUP	VIBRATION
1	443.64	10.17	C-Br stretch	Alkyl halides	M
2	1045.45	29.19	C-O stretch	Alcohols, COOH, Esters, ethers	S
3	1159.26	11.44	C-H Wag	Alkyl halides	M
4	1454.38	11.80	C-H bend	Alkanes	M
5	1647.26	4.62	-C=C- stretch	Alkenes	M
6	1743.71	10.55	C=O stretch	Esters, Sat Aliphatics	S
7	2354.20	21.84	C≡N stretch	Nitrile	M
8	2856.57	18.53	C-H stretch	Alkanes	M
9	2924.18	17.18	C-H stretch	Alkanes	
10	3381.33	46.95	O-H stretch	Alcohols, phenols	S,B

M-medium, S- strong, B-broad vibrational mode

CHAPTER FIVE

5.0 DISCUSSION

Phytochemicals are a large group of organic compounds found in plants, some of which include; alkaloids, saponins, flavonoids, sterols, tannins, cardiac glycosides and terpenoids. The biological activity of plants are mostly, manifestation of some of these phytochemicals detected in the crude extracts of *P. americana* seed and *C. odorata* leaf (Afolabi *et al*., 2007; Anyasor *et al,* 2011). The presence of Sterols, cardiac glycosides and terpenoids in all extracts of both plants was also reported by other researchers (Wang *et* a*l.,* 2010; Alisi *et al.*, 2011; Pahua-Ramos *et al.,* 2012). However, in another study, flavonoids, alkaloids and saponins were only slightly present in these plants (Ngozi *et al*., 2009; Arukwe *et al.,* 2012) and this could account for the absence of alkaloid in various solvent extracts of our samples, also absence of flavonoids in ethyl acetate and n-hexane extracts. Some of the phytochemicals detected have been reported to have good larvicidal activity, e.g. Phytosterol, β-sitosterol was reported as potent larvicide against *Aedes aegypti* (Rahuman *et al.,* 2008) while a terpenoid, hugorosenone isolated from *Hugonia castaneifolia* shows larvicidal activity against *Anopheles gambiae* (Baraza *et al.,* 2008). In another study, Gopieshkhanna and Kannabiran, (2007), showed that, saponins, phenols and flavonoids in plants have larvicidal activity and the larvicidal use of commercial saponin from bark of *Quillaja saponaria* was also reported (Pelah *et al*., 2002). Cardiac glycoside was demonstrated to have effect against larva and adult stages of the camel tick (Al-Rajhy *et al.*, 2003). These larvicidal studies suggest that, many of these phytochemicals play critical role in mosquito control programme, (Haq, 1999).

According to Kishore *et al.,* (2011), plant extracts mosquitocidal effect vary not only according to plant species, mosquito species and plant parts, but also to extraction methodology. Thus, in this study, our findings revealed that n-hexane (crude) extracts of both

plants are more potent than the ethanol and ethyl acetate (crude) extracts of the respective plants, showing potency variation due to change in solvent of extraction as reported by various scholars (Ghosh *et al*.,). The mortality increases as dose and exposure period increase in both plant with n-hexane extract of *Persea americana* seed as most potent, which affirms earlier larvicidal study (Leiti *et al*., 2009) carried out with hexane and methanol extract of *P. americana* against *Aedes aegypti* whereas the difference observed could be attributed to plant origin and mosquito species variation, (Shaalan *et al.,* 2005). Furthermore, the varying results in the two plants could be due to differences in levels of toxicity among the larvicidal active principal (phytochemicals) of each plant, (Rattan, 2010) and other scholars have demonstrated that extraction of active biochemical from the same plant depends upon the polarity of the solvents used, which is an important (larvicidal) response determinant (Haq *et al.,*1999; Yadav, *et al.,* 2002; Prabakar and Jebanesan, 2004; Mohan *et al.,* 2006; Raghavendra *et al.,* 2009; Rawani *et al.,* 2009; Kamaraj *et al* 2010; Mgbemena, 2010).

Consequently, n-hexane, a non- polar solvent, extract most of the oil constituents of the plants, many of which have been reported present, in both plants, and various scholars demonstrated remarkable larvicidal efficacy of these compounds found in other plants. Ding *et al.,* (2007), reported the presence of triterpenes in extract of *P. americana* seed and Nathan *et al.,* 2008, showed that triterpenes isolated from *Dysoxylum malabaricum* are potent larvicides. Similarly, β-sitosterol isolated from *Abutilon indicum* demonstrated good larvicidal activity (Rahuman *et al.,* 2008) and it was reported to be present in the n-hexane extract of *P. americana* seed (Leite *et a*l., 2009). While, Moses *et al.,* (2010), reported abundance of α-pinene, and germacrene D in *C. odorata,* and larvicidal studies of these compounds proved potent against *Aedes aegypti* and *Anopheles gambie* (Jantan *et al.,* 2005; Kiran and Devi, 2007).The possible mechanism of action of these essential oils could be due to surface materials (such as low molecular weight lipophilic compounds) entering the

tracheal system of the larvae, thus increasing tracheal flooding and chemical toxicity (Corbet *et al.*, 1995). The relatively higher toxicity found in the hexane extracts of both plants could also be due to lipophilic nature of the extracts of this solvent that increase turbidity at higher concentrations which cause oxygen depletion to the larvae, (Vinayagan *et al.*, 2008), and low molecular weight lipophilic compounds are soluble in the haemolymph after crossing the cuticle, thus provide another entry through the trachea, (Veal, 1996). Furthermore, several essential oil compounds have been demonstrated to interfere with basic biochemical, physiological and behavioural functions of insects when absorbed through skin which manifest delayed developments and morphological malformations (Khater *et al*, 2011).

The chromatographic techniques employed fractionated the most potent (crude) solvent extracts, showing increased potency in some fractions obtained from both plants. The most potent fraction; nHPa6 compared favourably with lactones isolated from *Hortonia floribunda* and tested against *Aedes aegypti* larvae (Ratnayake *et al.*, 2001) whereas nHPa5 show good larvicidal activity almost similar to larvicidal activity of a tetranortriterpenoid isolated from *Turrarea floribunda* and tested against *Anopheles gambie.* The relatively rapid action of the most potent fractions; nHPa6, nHCo6 and nHPa7 could be due to neurotoxic mode of action through octopamine receptor when absorbed through the skin (Enan, 2005). Similar response noticed in nHPa3 and nHPa2 could be due to an overlap in the constituents of the two fractions attributable to the eluent mixture (Leite *et al.*, 2009). The variations in the activity of the different fractions could be due to change in solvent polarity as the elution of the column progresses, which probably provide different bioactive constituents in the fractions obtained (Rahuman and Venkatesan, 2008; Ghosh *et al.*, 2012).This demonstrated that partial purification has increased the potency of the extracts and the most active compound(s) most probably lies within these fractions (Kishore *et al*. 2011).

The characterisation of most toxic fractions using GC/MS and FTIR spectroscopic techniques revealed the possible compounds and their functional groups respectively. The most dominant components have been reported as larvicidal compounds present as fatty acids and fatty methyl esters (FAME), in many plants (Kishore *et al.,* 2011). The larvicidal activity of fractionated *Piper nigrum* ethanol extract also identified oleic acid as the larvicidal compound (Santiago *et al.,* 2014) while Octadecanoic acid and n-hexadecanoic acid were the prominent larvicides against *Aedes albopictus* identified in *Pongamia pinnata* (Naik *et al.*,2014). In addition, Rahuman and Venkatesan (2008) showed that oleic and linoleic acids are toxic against *Aedes aegypti* larvae L., *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say. Furthermore, comparative study of the larvicidal activities of different fatty acids constituents against *Aedes aegypti* showed that oleic acid is the most effective (Tare and Sharma, 1991).

Farag, *et al*., (2011) reported that, the fatty acids and their esters contained in *Melia azedarach*, are mainly responsible for the insecticidal and growth inhibition activity against *Spodoptera littoralis*. Hexadecanoic acid and octadecanoic acid, were dominant fatty acid constituents present in *Senna fistula* (L.) and *S. portulacastrum* reported to have insect static and insecticidal activities against *Spodoptera frugiperda* (Barakat *et al*. 2004; Cantrell, 2011). Thus, Ramos-López *et al.* (2012), affirmed that the correlation of a compound and larval mortality opens the door for using fatty acids like, oleic acid, Hexadecanoic acid, and 2-hydroxy-methyl ester Oleic acid as natural larvicidal agents.

The FTIR technique identified functional groups in larvicidal components containing fatty acids and fatty methyl esters. These groups are thus probably the components responsible for larvicidal/repellent property exhibited by the fractions (Bansal and Sigh, 2005, Naik *et al*., 2014).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

i. All the solvent (ethanol, ethyl acetate and n-hexane) extracts of *Persea americana* seed and *Chromolaena odorata* leaf contained steroids, terpenoids and cardiac glycosides.

ii. n-hexane is the most potent solvent extract in both plants, followed by ethanol and then ethyl acetate extracts.

iii. The chromatographic fractionation of the most potent solvent (n-hexane) extracts showed increased larvicidal effect in some of the fractions nHPa6, nHPa7 nHCo3, nHCo6 and nHCo5.

iv. The GC/MS analyses of the most potent chromatographic fractions (nHPa6 and nHCo6) identified oleic acid as the most abundant component in the most potent (larvicidal) fractions of both plants.

v. The FTIR technique revealed the presence of carboxylic acid, carbonyl ester, alcohol, alkene, and alkyl halides in the most potent fractions of both plants.

6.2 Conclusion

The steroids, terpenoids and cardiac glycosides detected in all the solvents extracts of both plants could be responsible for the larvicidal efficacy of both plants. Larvicidal bioassay of *P. americana* seed and *C.odorata* leaf show that, n-hexane, ethyl acetate and ethanolic extracts of both plants have good larvicidal activity. nHPa6 and nHCo6 are the most potent fractions in *P. americana* and *C. odorata* respectively. The oleic acid could be synergistically responsible for the larvicidal efficacy of the two fractions (nHPa6, nHCo6).

6.3 Recommendations

The n-hexane, ethanol, and ethyl acetate extracts of *P.americana* seed and *Chromolaena odorata* leaf should be exploited for mosquito control. The oleic acid content of these plants should be explored and optimised for possible use as mosquito larvicide. There is a need for isolation and characterisation of active ingredients in ethanol and ethyl acetate extracts of these plants.

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of insecticide. *Journal of Economic Entomology, 18,* 265–7.

Adeyemi, O. O., Okpo, S. O. and Ogunti, O. O. (2002). Analgesic and anti-inflammatory effects of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae). *Fitoterapia, 73,* 375-380.

Afolabi, C., Akinmoladun, E.O., and Dan-Ologe, I.A. (2007). Phytochemical Constituents and Antioxidant properties of extracts from the leaves of *Chromolaena odorata*. *Scientific Research and Essay,* 2 (6), 191-194.

Ajao, A. T., Ajadi, T.S. and Oyelowo, M.S. (2011). Evaluation of Multiplicative Killing Effect of *C.odorata* extracts and β-lactam antibiotics against β-lactamase Producing bacteria, isolated from Selected Hospitals in Ilorin Metropolis. *Annals of Biological Research* Scholars Research Library, *2,* (4), 76-84.

Alaa, E. B., Balaña-Fouce, R., Sobeiha, A. K. and Hussein E. M. K. (1998). The biological activity of some chitin synthesis inhibitors against the cotton leafworm *Spodoptera littoralis* (Boisduval), (Lepidoptera: *Noctuidae). Bulletin of plant health, Pest, 24,* 499-506.

Alaba, A.O. (2005). *Malaria and Rural Household productivity in Oyo State*. (Doctoral dissertation, University of Ibadan, Nigeria).

Alisi, C., Ojiako, S. O. A., Osuagwu, C. G., and Onyeze, G. O. C. (2011). Free Radical Scavenging and *In-vitro* Antioxidant Effects of Ethanol Extract of the Medicinal Herb *Chromolaena odorata* Linn. *British Journal of Pharmaceutical Research,* 1(4), 141-155.

Al-Rajhy, D.H., Alahmed A.M., Hussein H.I. and Kheir, S.M. (2003). Acaricidal effects of cardiac glycosides, azadirachtin and neem oil against the camel tick, *Hyalomma dromedaril (Acari*: *Ixodidae*) *Pest Management Science, 59*(11): 1250-1254.

Amalraj, D. and Das, P.K. (1998). Estimation of predation by the larvae of *Toxorhynchites splendens* on the aquatic stages of *Aedes aegypti. Southeast Asian Journal of Tropical Medicine and Public Health*; *29,* 177-83.

Anees, A.M. (2008). Larvicidal activity of *Ocimum sanctum* Linn (*Labiatae*) against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say). *Parasitology Research,* 101, 1451-3.

Anon, (1983). *Important Weeds of the World* (3rd Edition). Leverkusen, Germany: Bayer A.G.

Anyasor, G.N., Aina, D.A.; Olushola M., and Aniyikaye, A.F. (2011). Phytochemical constituent, proximate analysis, antioxidant, antibacterial and wound healing properties of leaf extracts of *Chromolaena Odorata*. *Annals of Biological Research*; *2*(2): 441-451

Arukwe, U., Amadi, B.A., Duru, M.K.C., Agomuo, E.N., Adindu, E. A., Odika, P.C., Lele, K.C., Egejuru, L., and Anudike, J. (2012). Chemical Composition of *Persea*

americana Leaf, Fruit and Seed *International Journal of Research and Review in Applied Sciences;* 11 (2).

Ascher, K. R. S., Melamed-Madjar, V., Nemny, N. E. and Tam, S., (1987). The effect of benzoylphenylurea moulting inhibitor of larvae and eggs of European corn borer, *Ostrinia nubialis* Hb. (Lepidoptera: pyralidae). *Journal of Plant Diseases and Protection, 7*(9), 231-237

Atindehou, M., Lagnika, L., Guérold, B., Strub J. M., Zhao M.; Van Dorsselaer A., Marchioni E., Prévost G., Haikel Y., Taddéi C., Sanni A., and Metz-Boutigue, M. (2013). Isolation and Identification of two antibacterial agents from *Chromolaena odorata* (L) active against four diarrheal strains. *Advances in Microbiology, 3*, 115-121.

Ayyanar, M., and Ignacimuthu, S. (2009). Herbal medicines for wound healing among tribal people in Southern India: Ethnobotanical and Scientific evidences. *International Journal of Applied Research in Natural Products, 2*(3), 29-42.

Bansal, S. K. and Singh, K.V. (2005) *Determination of larvicidal potential of active principle(s) of Solanum xanthocarpum against important mosquito vectors*. Unpublished manuscript.

Barakat A. A., El-Mahy, A. S. Omaima, K. Moustafa, K. O. Mansour, F. A. and El-hadek. K. M. (2004). Biological effect of *Cassia fistula* (L.) seeds against the cotton leafworm *Spodoptera littoralis* (Boisd.) with special reference to chemical constituents. *Bulletin of Entomology Society*, Egypt, Economic Series, 30, 1-14.

Baraza, L.D., Joseph, C.C., Munissi, J.J.E., Nkunya, M.H.H., Arnold, N., and Porzel, A., (2008). Antifungal rosane diterpenes and other constituents of *Hugonia castaneifolia*. *Phytochemistry*, 6, 200-5.

Barrett, A. D. and Higgs S. (2007). Yellow fever: a disease that has yet to be conquered. *Annual Review of Entomology; 52*, 209–29.

Bennett, F.D. and Rao, V.P., (1968). Distribution of an introduced weed *Eupatorium odoratum* L. (Compositae) in Asia and Africa and possibility of its biological control. *PANS* (C) 14: 277-281.

Bhatt, S., Gething P.W. and Brady, O.J. (2013). The global distribution and burden of dengue. *Nature, 496* (7446), 504–7.

Brai, B., Odetola, A.A., Agomo, P.U. (2007). Effects of *Persea americana* leaf extracts on body weight and liver lipids in rats fed hyperlipidaemic diet. *African Journal of Biotechnology, 6*(8), 1007–1011

Brown, A.W.A., (1986) Insecticide resistance in mosquitoes: a pragmatic review. *Journal of American Mosquito Control Association*, 2:123–40

Cantrell, C.L., ALI, A., DUKE, S.O, and Khan, I., (2011). Identification of mosquito biting deterrent constituents from the Indian folk remedy plant *Jatropha curcas*. *Journal of Medical Entomology*, 48(4):836-4.

Chahabra, M, Mittal V, Bhattacharya, D., Rana, U., and Lal, S. (2008). Chikungunya fever: a re-emerging viral infection. *Indian Journal of Medical Microbiology, 26* (1): 5–12.

Chansang, U., Zahiri, N.S., Bansiddhi, J., Boonruad, T., Thongsrirak, P., and Mingmuang, J., *et al*. (2005). Mosquito larvicidal activity of crude extracts of long pepper (*Piper retrofractum* vahl) from Thailland. *Journal of Vector Ecology, 30,* 195-200.

Chariandy, C.M., Seaforth, C.E., Phelps, R.H., Pollard, G.V., Khambay, B.P.S., (1999). Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. *Journal of Ethanopharmacy, 64*, 265–270.

Chowdhury, N., Ghosh, A., and Chandra, G. (2008). Mosquito larvicidal activities of *Solanum villosum* berry extract against the Dengue vector *Stegomyia aegypti*. *BMC Complementary Alternative Medicine, 8,* 10.

Clements, A. (1992). *The biology of mosquitoes: Development, Nutrition and Reproduction*. London: Chapman & Hall.

Collins, F.H., Paskewitz, S.M., (1995). Malaria: current and future prospects for control. *Annual Review on Entomology, 40, 195–219.*

Collins, W.E. (2012). *Plasmodium knowlesi*: a malaria parasite of monkeys and humans; *Annual Review of Entomology, 57,* 107-21.

Corbet, S. A., Danahar, C.W., King, V., Chalmers, C. L., and Tiley, C. F. (1995). Surfactant-enhanced essential oils as mosquito larvicides. *Entomologia Experimentalis et Applicata*. 75, 229–236.

Cruttwell Mc Fadyen, R. E. (1991). The Ecology of *Chromolaena odorata* in the Neotropk.s. *ln* R. Muniappan & P. Ferrar (Eds.), Ecology and Management of *Chromolaena odorata.* Biotropic Special Publication, 44, 1-7.

Das, P.K., Tyagi, B.K, and Somachari, N.V. (1986). Efficacy of Arosurf, a monomolecular surface film in controlling *Culex quinquefasciatus* Say, *Anopheles stephensi* Liston and *Aedes aegypti* (L.). *Indian Journal of Medical Research, 83*:271-276.

Dieng, H., Boot, M., Tuno, N., Tsuda, Y., and Takagi, M., (2002). A pehpeiensis as laboratory and field evaluation of *Macrocyclops distinctus, Megacyclops viridis* and Mesocyclops control agents of the dengue vector *Aedes albopictus* in a peridomestic area in Nagasaki, *Japan Medical and Veterinay Entomology, 16* 285-91.

Ding, H., Chin, Y. W., Kinghorn, A. D., and D'Ambrosio, S. M. (2007). Chemopreventive characteristics of avocado fruit. *Seminars in Cancer Biology, 17*: 386–394.

Edmund, C. Jaeger, (1959). *A Source-Book of Biological Names and Terms* (125-130). Springfield: Thomas.

Ejele, A. E., Akpan, I.O., Ogukwe. C.E., Onyeocha, V.O., and Ukiwe, L.N. (2012) Bioassay-guided isolation and partial characterization of an antisickling compound from *Enantia chlorantha*. *International Research Journal of Biochemistry and Bioinformatics, 2*(7), 149-154.

El-Guindy, M. A., Abdel-Sattar, M. M., Dogheim, S. M., Madi, S. M. and El Assar, M. R. S., (1982). Laboratory evaluation of the insect growth regulator dimilin (TH6040) against susceptible and resistant strains of *Spodoptera littoralis* (Boisd.), *International Pest Control, 25,* 48-51.

Enan, E.E. (2005). Molecular and pharmacological analysis of an octopamine receptor from American cockroach and fruit fly in response to plant essential oils. *Archives of Insect Biochemistry and Physiology*, 59, 161-171.

Fang, J. (2010). Ecology: A world without mosquitoes. *Nature*, *466* (7305), 432–4.

Farag, M., Ahmed, M. H. M., Yousef, H. and Abdel-Rahman, A. A. H. (2011). Repellent and insecticidal activities of *Melia azedarach* L. against cotton leafworm *Spodoptera littoralis*. Zeitschrift fuer Naturforschung. *66*c, 129-135.

Feitelson, J.S., Payne, J., Kim, L. (1992). *Bacillus thuringiensis*: insects and beyond. *Nature Biotechnology*. *10*, 271-275.

Finlay, C.J. (2012). The Mosquito Hypothetically Considered as the Transmitting Agent of Yellow Fever. *Medical Review*, *14* (1): 56–9.

Finney, D.J. (1971). *Probit analysis*, (3rd edition) Cambridge: Cambridge University Press.

Foster, W. and Walker, E. (2002). *Medical and Veterinary Entomology*. San Diego: Academic Press. (pp 203-262)

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

Goma, L. K. H. 1966 *Mosquitoes*. Hutchinson. pp 22-30

Gomez-Lopez, V. M. (1999). Characterization of avocado (*Persea americana* Mill.) varieties of low oil content. *Journal of Agricultural and Food Chemistry*; 47(7); 2707–2710.

Goodell, P.B. (2004) *Insecticide resistance management and quality cotton*. U.C. State-wide, Integrated Pest Management Project.

Gopieshkhanna, V. and Kannabiran, K. (2007). Larvicidal effect of *Hemidesmus indicus* and *Gymnema sylvestre* against *Culex quinquifaciatus* mosquito larvae. *African Journal of Biotechnology*, *6*(3): 307-311.

Govindarajan, M., Sivakumar, R., Amsath, A., Niraimathi, S. (2011). Mosquito larvicidal properties of *Ficus benghalensis* L. (Family: *Moraceae*) against Culex tritaeniorhynchus Giles and *Anopheles subpictus* Grassi. *Asian Pacific Journal of Tropical Medicine*, 505-509.

Govindarajan, M. (2009). Bioefficacy of *Cassia fistula* Linn.(Leguminosae) leaf extract against chikungunya vector, *Aedes aegypti* (Diptera: Culicidae). *European Review for Medical and Pharmacological Sciences*, *13*, 99-103.

Govindarajan, M., Jebanesan, A., Pushpanathan, T. (2008). Larvicidal and ovicidal activity of *Cassia fistula* leaf extract against filarial and malarial vector mosquitoes. *Parasitology Research*, *102*, 289-292.

Grossman, G. L. and James, A. A. (1993). The salivary glands of the vector mosquito, *Aedes aegypti*, express a novel member of the amylase gene family. *Insect Molecular Biology* 1 (4), 223–232.

Gubler, D. (2010). Dengue and dengue hemorrhagic fever. *Clinical Microbiology Review, 11* (3), 480–96.

Guzman, M.G., Halstead, S.B., Artsob, H. (2010). Dengue: a continuing global threat; *Nature Reviews Microbiology, 8* (12 Suppl), S7–S16.

Haas, P., (1952). Variations in the compositions of avocadoseed *California Avocado Society proceedings Yearbook,* 3: 56-59.

Hanson, S. M. and Craig, G. B. (1995). *Aedes albopictus* (Diptera: Culicidae) eggs: field survivorship during northern Indiana winters. *Journal of Medical Entomology, 32* (5), 599–604.

Haq, E., Nadi, E.A.A.H. and Zaitoon, A.A. (1999). Toxic and growth retarding effects of three plant extracts on *Culex pipiens* larvae (Diptera: Culicidae). *Phytotherapy Research,* 13: 388-392.

Harborne, J.B. (1984). *Phytochemical methods*: *A guide to modern techniques in plants analysis*; 2nd Edition, chapman and Hall, London; PP. 1-10, 100-117.

Harzsch, S. and Hafner, G. (2006). Evolution of eye development in arthropods: Phylogenetic aspects. *Arthropod Structure and Development, 35* (4): 319–340.

Hawley, W. A., Pumpuni, C. B., Brady, R. H. and Craig, G. B. (1989). Overwintering survival of *Aedes albopictus* (Diptera: Culicidae) eggs. *Indian Journal of Medical Entomology 26* (2), 122–129.

Higgs, S. (2004). Differential modulation of murine host immune response by salivary gland extracts from the mosquitoes *Aedes aegypti* and *Culex quinquefasciatus*. *Medical and Veterinary Entomology, 18* (2), 191–199

Hopkins, D.R. (2013). Disease Eradication. *New England Journal of Medicine, 368* (368), 54–63. http://edis.ifas.ufl.edu/IN077.

Huang, J., Walker, E. D., Vulule, John, Miller and James, R. (2006). Daily temperature profiles in and around Western Kenyan larval habitats of *Anopheles gambiae* as related to egg mortality. *Malaria Journal, 5,* 87.

Idris, S., Ndukwe G.I. and. Gimba C.E. **(**2009). Preliminary Phytochemical Screening and Antimicrobial Activity of Seed Extracts of *Persea americana* (Avocado Pear**).** *Bayero Journal of Pure and Applied Sciences, 2(1),*173 – 176.

Imafidon, K.E., and Amaechina, F.C. (2010). Effects of aqueous seed extract of *Persea americana* M. on blood pressure and lipid profile in hypertensive rats. *Advanced Biological Research; 4*(2), 116–121.

Jantan, I., Yalvema, M.F., Ahmad, N.W., Jamal, J.A. (2005). Insecticidal activities of the leaf oils of eight *Cinnamomum* species against *Aedes aegypti* and *Aedes albopictus. Pharmceutical Biology, 43,* 526-32.

Jenkins, D.W. (1964) Pathogens, parasites and predators of medically important arthropods. *Bulletin of WHO, 30,* supplementary, 28-37.

Kamaraj, C., and Rahuman, A.A. (2010). Larvicidal and adulticidal potential of medicinal plant extracts from south India against vectors. *Asian Pacific Journal of Tropical Medicine, 3,* 948-53.

Kamaraj, C., Rahuman, A.A., Bagavan, A., Abduz Zahir, A., Elango, G., and Kandan, P. (2010). Larvicidal efficacy of medicinal plant extracts against *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). *Tropical Biomedicine, 27,* 211-9.

Karmegan N, Sakthivadivel M, Anuradha V, and Daniel T. (1997). Indigenous plant extracts as larvicidal agents against *Culex quinquefasciatus* Say. *Bioresource Technology, 5,* 137-40.

Karmegan, N., Sakthivadivel, M., Anuradha, V., and Daniel, T. (1997). Indigenous plant extracts as larvicidal agents against *Culex quinquefasciatus* Say. *Bioresource Technology, 5,* 137-40.

Karunamoorthi, K., Mulelam, A., and Wassie, F. (2009) Assessment of knowledge and usage of custom of traditional insect/ mosquito repellent plants in Addis Zemen Ethiopia. *Journal of Ethnopharmacology,* 12: 1, 49-53.

Kaushik, R. and Saini P. (2008). Larvicidal activity of leaf extract of *Millingtonia hortensis* (Family: *Bignoniaceae*) against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti. Journal of Vector Borne Disease,* 45, 66-9.

Kawagishi, H., Fukumoto, Y., Hatakeyama, M., He, P., Arimoto, H., Matsuzawa, T., Arimoto, Y.,Suganuma, H., Inakuma, T. and Sugiyama, K. (2001). Liver injury suppressing compounds from avocado (*Persea americana*). *Journal of Agriculture and Food Chemistry*, 49, 2215-2221.

Khater, H.F., Hanafy, A.M., Abdel- Mageed, A.D., Ramadan, M.Y., & El- Madawy, R.S. (2011). The insecticidal effect of some Egyptian plant oils against *Lucilia sericata* (Diptera: Calliphoridae). *International Journal of Dermatology,* 50 (2), 187–194.

Kim, S., Heung Chul, Terry A. Klein, Takhampunya, R. Evans, B. P., Mingmongkolchai, Ampornpan Kengluecha, John Grieco, Penny Masuoka, Myung-Soon Kim, Sung-Tae Chong, Jong-Koo Lee, and Won-Ja Lee. (2011). Japanese Encephalitis Virus in Culicine Mosquitoes (Diptera: Culicidae) Collected at Daeseongdong, a Village in the Demilitarized Zone of the Republic of Korea. *Journal of Medical Entomology 48*(6), 1250-1256.

Kiran, S.R. and Devi, P.S. (2007). Evaluation of mosquitocidal activity of essential oil and sesquiterpenes from leaves of *Chloroxylon swietenia* DC. *Parasitology Research,* 101, 413-8.

Kishore, N., Mishra B.B., Tiwari V.K., Tripathi, V. A. (2011). Review on natural products with mosquitocidal potentials; Opportunity, challenge and scope of natural products in medicinal chemistry. Kerala: *Research Signpost*, 335-65.

Kosova, J. (2003). Longevity Studies of *Sindbis Virus* Infected *Aedes Albopictus*. All Volumes (2001–2008). http://digitalcommons.unf.edu/ojii_volumes/94

Krishnan, K., Senthilkumar, A., Chandrasekaran, M., and Venkatesalu, V. (2007) Differential larvicidal efficacy of four species of *Vitex* against *Culex quinquefasciatus* larvae. *Parasitology Research, 10,* 1721-3.

Kumar, A., Dutta, G.P., (1987). Indigenous plant oils as larvicidal agent against Anopheles stephensi mosquitoes. *Current Science, 56,* 959–960

Kunow, M.A. (2003). *Plants with uses similar to roys*. In: Kunow MA, editor. Maya medicine: traditional healing in Yucatan. Albuquerque: University of New Mexico Press. ph. 128.

Lahariya, C., Pradhan, S.K. (2006). Emergence of chikungunya virus in Indian subcontinent after 32 years: A review. *Journal of Vector Borne Diseases, 43* (4), 151–60.

Larson, L.L. (2001). Mergers, regulation, resistance management, and new product approaches: The odyssey continues for industry, Informal Conference during the Annual Meeting of Entomological Society of America; San Diego, CA, http://www.entsoc.org/Protected/AMT/AMT27/Text/Other/ConferenceReport.

Lavanya, G. & Brahmaprakash, G. P (2011) Phytochemical Screening and Antimicrobial Activity of Compounds from Selected Medicinal and Aromatic Plants. *International Journal of Science and Nature.* 2*(2):* 287- 291

Leisnham, P. (2010). Taking a bite out of mosquito research. University of Maryland. Enst.umd.edu Retrieved on 2013-04-01

Leite, G., S Brito, Aguiar, C.R., Sâmia, R.N., José Júlio, B., Sidrim, C., Medeiros, L. Bertini, Selene Maia de Morais and. Gadelha Rocha, M. F (2009). Chemical composition, toxicity and larvicidal and antifungal activities of *Persea americana* (avocado) seed extracts *Revista da Sociedade Brasileira de Medicina Tropical 42*(2), 110-113.

Lopez, C.C., Scorsetti, A.C., Marti, G.A., Garcia, J.J. (2004). Host range and specificity of an Argentine an isolate of the aquatic fungus *Leptolegnia chapmanii* (*Oomycetes: Saprolegniales*), a pathogen of mosquito larvae (*Diptera: Culicidae*). *Mycopathologia, 158*: 311-5.

Lu, Q.Y., Arteaga, J.R., Zhang, Q., Huerta, S., Go V.L and Heber, D. (2005). Inhibition of prostate cancer cell growth by an avocado extract: role of lipid-soluble bioactive substances. *Journal of Nutritional Biochemistry*, 16, 23-30.

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

Mathivanan, T., Govindarajan, M., Elumalai, K., Krishnappa, K., and Ananthan, A. (2010). Mosquito larvicidal and phytochemical properties of *Ervatamia coronaria* (Family: *Apocynaceae*); *Journal of Vector Borne Diseases, 47,* 178-180.

McKinley, N., Kijima, S., Cook, G. and Sherrod, D. (2002). Avaunt (indoxacarb): A new mode of action insecticide for control of several key orchard pests; *Proceedings of 76th Annual Western Orchard Pest & Disease Management Conference;* 2.

McNeill, J. R. (2004). Yellow Jack and Geopolitics: Environment, Epidemics, and the Struggles for Empire in the American Tropics, 1650–1825. *OAH Magazine of History* 18(3), 9-13.

Mgbemena, I.C. (2010). Comparative evaluation of larvicidal potentials of three plant extracts on *Aedes aegypti*. *Journal of American Society for Horticulture*; *6* : 435-40.

Miyagi, I., Toma, T., and Mogi, M. (1992). Biological control of container breeding mosquitoes, *Aedes albopictus* and *Culex quinquefasciatus*, in a Japanese island by release of *Toxorhynchites splendens* adults. *Medical and Veterinary Entomology, 6,* 290-300.

Mohammed, A.A. (2003). Study of larvivorous fish for malaria vector control in Somalia. *East Mediterranean Health Journal, 9,* 618-26.

Mohan, L., Sharma P. and Srivastava, C.N. (2005). Evaluation of *Solanum xanthocarpum* extracts as mosquito larvicides. *Journal of Environmental Biology;* 26(2): 399-401.

Mohan, L., Sharma, P., and Srivastava, C.N. (2006). Evaluation of *Solanum xanthocarpum* extract as a synergist for cypermethrin against larvae of filarial vector *Culex quinquefasciatus* (Say). *Entomology Research, 36,* 220-5.

Moses, S.O., Ogundajo, A., Kamil, O. Y., Lajide, L., Heather, E. V, Jessika A. T., and William, N. S. (2010). Chemical Composition and Bioactivity of the Essential Oil of *Chromolaena odorata* from Nigeria. *Record of Natural Product;* 4:1 72-78

Muesebeck, C. F. W. (1952). Mosquitoes Biology. *Entomology Society Proceedings*. 42:91-93.

Mullen, G., and Durden, L. (2009). *Medical and Veterinary Entomology*; London: Academic Press. Pp 23-38

Mutebi, J. P. and A. D. Barrett (2012). The epidemiology of yellow fever in Africa; *Microbes Infect, 4* (14), 1459–1468.

Naik, B. R.., Gowreeswari, G. S., Singh, Y., Satyavathi, R., Daravath, S. S., and Ramachandra, R. P. (2014**).** Bio-Synthesis of Silver Nanoparticles from Leaf Extract of *Pongamia pinnata* as an Effective Larvicide on Dengue Vector *Aedes albopictus* (Skuse) (Diptera: Culicidae). *Advances in Entomology***,** *2,* 93-101

Nathan, S.S., Hisham, A., and Jayakumar, G. (2008). Larvicidal and growth inhibition of the malaria vector *Anopheles stephensi* by triterpenes from *Dysoxylum malabaricum* and *Dysoxylum beddomei*. *Fitoterapia, 79* (2), 106-111.

Nayak, B.S., Raju, S.S. and Chalapathi Rao, A.V. (2008). Wound healing activity of *Persea americana* (avocado) fruit: a preclinical study on rats. *Journal of Wound Care,* **17**: 123-126.

Ngozi, M., Ikewuchi C. J. and Ikewuchi C. C. (2009). Chemical Profile of *Chromolaena odorata* (L) Leaves. *Pakistan Journal of Nutrition,* 8 (5): 521-524.

Nimesh, Gupta and P.V. Lakshmana Rao (2011). Transcriptomic profile of host response in Japanese encephalitis virus infection. *Virology Journal, 8* (92): 92.

Noudogbessi, J-P., Kossou, D. and Sohounhloué, D.C.K. (2006). Composition chimique et Propriétés Physico-Chimiques des Huiles Essentielles de *Pimenta racemosa* (Miller)

et de *Chromolaena odorata* (L. Robinson) Acclimatées au Bénin. *J. Soc. Ouest-Afr. Chim.*, 026, 11–19

Nur Jannah, M. H., Mahmood, A. A., Sidik, K. and Salmah, I. (2006). Cytoprotective effects of honey in combination with aqueous and ethanol extracts from *Chromolaena odorata* L (*Eupatorium odoratum*) in rats. *Jummec*; 9:(1).

O'Neill, J. (1997). *Mallis Handbook of Pest Control* (2nd edition): pp836-880.

Ogbonnia, S. O., Mbaka, G. O., Anyika E. N., Osegbo O. M., and Igbokwe, N. H. (2010). Evaluation of acute toxicity in mice and subchronic toxicity of hydroethanolic extract of *Chromolaena odorata* (L.) King and Robinson (Fam. Asteraceae) in rats. *Agriculture and Biology Journal of North America, 1(5),* 859-865.

Ogunbiyi, A., Aruboula, E. and Soladoye, A. O. (2008). Analgesic, anti-inflammatory and antipyretic activities from flavonoid fractions of *Chromolaena odorata*. *Journal of Medicinal Plants Research*; 2(9), 219-225.

Ojewole, J.A., and Amabeoku, G.J. (2006). Anticonvulsant effect of *Persea americana* Mill (Lauraceae) leaf aqueous extract in mice. *Phytotheraphy Research, 20* (8): 696-700.

Ortiz, M.A., Dorantes, A.L., Galindez, M.J., and Cárdenas, S.E., (2004). Effect of a novel oil extraction method on avocado (*Persea americana* Mill) pulp microstructure. *Plant Foods for Human Nutrition, 59(1):11–14.*

Ottesen, E.A., Hooper, P.J., Bradley, M., and Biswas, G. (2008). The Global Programme to Eliminate Lymphatic Filariasis: Health Impact after 8 Years. In De Silva, Nilanthi, *PLOS Neglected Tropical Diseases* 2 (10): e317

Outland, K. (2005). New Treatment for Elephantitis: Antibiotics. *The Journal of Young Investigators*, 13.

Owolabi, M.A., Jaja, S.I., and Coker, H.A. (2005). Vasorelaxant action of aqueous extract of the leaves of *Persea americana* on isolated thoracic rat aorta. *Fitoterapia, 76* (6), 567-573.

Oyewole, I.O., Ibidapo, C.A., Okwa, O.O., Oduola, A.O., Adeoye, G.O., Okoh, H.I., and Awolola, T.S. (2010). Species Composition and Role of *Anopheles* Mosquitoes in Malaria Transmission Along Badagry Axis of Lagos Lagoon, Lagos, Nigeria. *International Journal of Insect Science* vol. 2, 51–57.

Ozolua, R.I., Anaka, O.N., Okpo S.O., and Idogun S.E. (2009). Acute and sub-acute toxicological assessment of the aqueous seed extract of *Persea americana* Mill (Lauraceae) in rats. *African Journal of Traditional Complementary and Alternative Medicine* 6(4):573–578

Pahua-Ramos, M. E., Ortiz-Moreno A., Chamorro-Cevallos, G., Hernández-Navarro, M. D., Garduño-Siciliano L., Necoechea-Mondragón, H., and Hernández-Ortega, M. (2012). Hypolipidemic Effect of Avocado (Persea americana Mill) Seed in a Hypercholesterolemic Mouse Model. *Plant Foods for Human Nutrition, 67,*10–16.

Panda, D., Dash, S. K., and Dash, G. K. (2010). Qualitative phytochemical analysis and investigation of anthelminthic and wound healing potentials of various extracts of *Chromolaena odorata* L. collected from the locality of Mohuda Village, Berhampur (south Orissa). *International Journal of Pharmaceutical Sciences Review and Research*, 1(2), Article 023.

Pelah, D., Z. Abramovich, A. Markus and Z. Wiesman, 2002.The use of commercial saponin from Quillaja saponaria bark as a natural larvicidal agent against *Aedes aegypti* and *Culex pipiens*. *Journal of Ethnopharmacology, 81*(3), 407-409.

Petersen, J.J. (1982). Current status of nematodes for biological control of insects. *Parasitology.* 84: 177-204.

Petersen, J.J. (1985) Nematodes as biological control agents: Pt I, Mermithidae. *Advances in Parasitology,* 14: 307-34.

Phan, T.T., Wang, L., See, P., Grayer, R..J., Chan, S.Y., and Lee, S.T. (2001). Phenolic Compounds of *Chromolaena odorata* Protect Cultured Skin Cells from Oxidative Damage: Implication for Cutaneous Wound Healing. *Biological and Pharmaceutical Bulletin, 24* (12), 1373—1379.

Poopathi, S., and Baskaran, G. (2001). Efficacy of *Bacillus thuringiensis* serovar *israelensis* against *Bacillus sphaericus* resistant and susceptible larvae of *Culex quinquefasciatus; Journal of Biological Control,* 15:85 – 92.

Powers, A.M., and Logue, C.H. (2007). Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus; *Journal of General Virology, 88* (Pt 9), 2363–77.

Prabhakar, K., Jebanesa, A. (2004). Larvicidal efficacy of some cucurbitacious plant leaf extracts against *Culex quinquefasciatus. Bioresource Technology*, 9, 113-4.

Pushpanathan,T., Jebanesan, A., and Govindarajan, M. (2008). The essential oil of *Zingiber officinale* Rosc (*Zingiberaceae*) as a mosquito larvicidal and repellent agent against the filarial vector *Culex quinquefasciatus* (Say) (Diptera: Culicidae). *Parasitology Research*; *102*, 1289-1291.

Raghavendra, K., Singh, S.P., Subbarao, S.K. and Dash, A.P. (2009). Laboratory studies on mosquito larvicidal efficacy of aqueous & hexane extracts of dried fruit of *Solanum nigrum* Linn. *Indian Journal of Medical Research, 130,* 74-7.

Rahuman A.A., and Venkatesan, P. (2008). Larvicidal efficacy of five cucurbitaceous plant leaf extracts against mosquito species. *Parasitology Research, 103,* 133-9.

Rahuman A.A., Gopalakrishnan G, Venkatesan P, and Geetha K. (2007). Larvicidal activity of some Euphorbiaceae plant extracts aginst *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research, 102,* 867-73.

Ralph, H. (2008). *Family Culicidae Meigen, 1818*: Mosquito Taxonomic Inventory, City. Pp 5-9.

Ramos-Jerz, M.R. (2007). Phytochemical analysis of avocado seeds (*Persea americana* Mill., c.v. Hass). *Phytochemistry*, 65:955-962

Ramos-López, M. A., González-Chávez, M. M., Cárdenas-Ortega, N. C., Zavala-Sánchez M.A., and Pérez, G. S. (2012). Activity of the main fatty acid components of the hexane leaf extract of *Ricinus communis* against *Spodoptera frugiperda*. *African Journal of Biotechnology, 11*, 4274-4278.

Ramsewak , R. S., Nair, M.G. Murugesan, S. Mattson W. J. and Zasada. J. (2001). Insecticidal fatty acids and triglycerides from *Dirca palustris. Journal of Agriculture and Food Chemistry*. *49*, 5852-5856.

Ratnayake, R.., Karunarantne, V., Ratnayake, B.M., Kumar, V., John, K.2001 Two New Lactones with Mosquito Larvicidal Activity from Three *Hortonia* Species. *Journal of Natural Product, 64* (3): 376–378.

Rattan, R.S. (2010). Mechanism of action of insecticidal secondary metabolites of plant origin; *Crop Protection*, *29*, 913-20.

Rawani, A., Ghosh, A., Chandra, G. (2010). Mosquito larvicidal activities of *Solanum nigrum* L. leaf extract against *Culex quinquefasciatus* Say. *Parasitology Research, 107,* 1235-40.

Rawani, A., Mallick Haldar, K., Ghosh, A., and Chandra, G. (2009). Larvicidal activities of three plants against filarial vector *Culex quinquefasciatus* Say (Diptera: *Culicidae*). *Parasitology Research*, *105*, 1411-7.

Redwane, A., Lazrek, H.B., Bouallam, S., Markouk, M., Amarouch, H., Jana, M. (2002). Larvicidal activity of extracts from *Quercus lusitania* var. infectoria galls (Oliv.). *Journal of Ethnopharmacology*, 79, 261−263.

Ribeiro, J. M. and Francischetti, I. M. (2003). Role of arthropod saliva in blood feeding, sialome and post-sialome perspectives, *Annual Review of Entomology, 48,* 73–88.

Richards, O.W., and Davies, R.G. (1977). *General Textbook of Entomology*: *Volume 1: Structure, Physiology and Development Volume 2: Classification and Biology*. Berlin: Springer.

Roark, R.C. (1947). Some promising insecticidal plants. *Economic Botany, 1*: 437-45.

Roberts, D.W., and Strand, A. (1977). Pathogens of medically important arthropods. *Bulletin WHO, 55* (Suppl.I), 419.

Rodenhuis-Zybert, I.A., Wilschut, J., Smit J.M., (2010). Dengue virus life cycle: viral and host factors modulating infectivity; *Cellularand Molecular Life Science. 67* (16), 2773–86.

Rodriguez, J., Garcia, I.G., Diaz, M., Avila, I.G., and Sanchez, J.E. (2003). Pathogenic effect of the parasite nematode *Strelkovimermmis spiculatus* (Nematoda Mermithidae) in larvae of mosquito *Culex quinquefasciatus* (Diptera: Culicidae) under laboratory conditions in Cuba. *Journal of Medical Entomology,* 55: 124-5.

Roll Back Malaria (2000) Abuja Declaration and Plan of Action

Romi, R., Severini, F., and Toma, L. (2006). Cold acclimation and overwintering of female *Aedes albopictus* in Roma. *Journal of the American Mosquito Control Association 22* (1): 149–151.

Rossignol, P. A. and Lueders, A. M. (1986). Bacteriolytic factor in the salivary glands of *Aedes aegypti*. *Comparative Biochemistry and Physiology, B 83* (4), 819–822.

Salazar, M.J., El-Hafidi, M., Pastelin, G., Ramírez-Ortega, M.C., and Sánchez-Mendoza, M.A., (2005). Effect of an avocado oil-rich diet over an angiotensin II-induced blood pressure response. *Journal of Ethnopharmacology, 98*, 335-338.

Samidurai, K., Jebanesan, A., Saravanakumar, A., Govindarajan, M., and Pushpanathan, T. (2009). Larvicidal, ovicidal and repellent activities of *Pemphis acidula* (Lythraceae) against filarial and dengue vector mosquitoes. *Academic Journal of Entomology, 2*, 62-66.

Santiago, V.S., Grace, R. A., and Irene, M., (2014) *Aedes aegypti* larvicide from the ethanolic extract of *Piper nigrum* black peppercorns *Natural Product Research, 29(5)* 441-443.

Sawabe, K.; and Moribayashi, A. (2000). Lipid utilization for ovarian development in an autogenous mosquito, Culex pipiens molestus (Diptera: Culicidae); *Journal of Medical Entomology, 37* (5): 726–731.

Saxena, R.C., Dixit, O.P., and Sukumaran, P. (1992). Laboratory assessment of indigenous plant extracts for anti-juvenile hormone activity in *Culex quinquefasciatus. Indian Journal of Medical Research, 95,* 199-204.

Saxena, S.C., and Sumithra, L., (1985). Laboratory evaluation of leaf extract of new plant to suppress the population of malaria vector Anopheles stephensi Liston (Diptera: Culicidae). *Current Science, 54*, 201–202.

Senthilnathan, S., Choi M.Y., Seo H.Y., Paik C.H., Kalaivani, K., and Kim J.D., (2008). Effect of azadirachtin on acetylcholinesterase (AChE) activity and histology of the brown planthopper *Nilaparvata lugens* (Stål). *Ecotoxicology and Environmental Safety, 70*(2), 244-50.

Service, M. W. (1986) Lecture Notes on Medical Entomology; pp 1-86.

Shaalan, E.A., Canyonb, D., Younesc, M.W.F., Abdel-Wahaba, H. and Mansoura, A.H. (2005). A review of botanical phytochemicals with mosquitocidal potential; *Environment International, 3*: 1149-66.

Sharma, P., Mohan, L. and Srivastava, C.N. (2004). Larval susceptibility of *Ajuga remota* (Diptera: Culicidae) against *anopheline* and *culicine* mosquitoes. *Southeast Asian Journal of Tropical Medicine Public Health*, 35(3), 608-610.

Sharma, P., Mohan, L., and Srivastava, C.N., (2006). Phytoextract-induced developmental deformities in malaria vector. *Bioresource Technology, 97*, 1599-604.

Simmons, C.P., Farrar J.J., Nguyen, V., and Wills, B. (2012). Dengue; *New England Journal of Medicine, 366* (15), 1423–32.

Singh, R. K., Dhiman, R. C. and Singh, S. P. (2003). Laboratory studies on the predatory potential of dragon-fly nymphs on mosquito larvae; *Journal of Communicable Diseases* **35** (2): 96–101.

Singh, R.K., Dhiman, R.C., and Mittal, P.K. (2007). Studies on mosquito larvicidal properties of *Eucalyptus citriodora* Hook (family- *Myrtaceae*). *Journal of Communicable Diseases, 39,* 233-6.

Sofowora, A. (1993). *Medicinal plants and Traditional medicine in Africa*: Spectrum Books Ltd, Ibadan, Nigeria.

Solomon, T. (2006). Control of Japanese Encephalitis, within our grasp? *New England Journal of Medicine, 355* (9), 869–871.

Song, Y. and Barlow, P. J. (2004). Antioxidant activity and phenolic content of selected fruit seeds. *Food Chemistry, 88*(3), 411–417

Spielman, A.,and D'Antonio, M., (2001). *Mosquito a natural history of our most persistent and deadly foe*. New York: Hyperion. Pp 15-16.

Srivastava, H.C. and Sharma, S.K. (2000). Chloroquine resistant *Plasmodium falciparum* in migrant population. *Indian Journal of Malariology*, 37: 39-42.

Sukanya, S.L., Sudisha, P. J., Hariprasad, S.R., Niranjana, Prakash, H.S., and. Fathima, S.K., (2009). Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. *African Journal of Biotechnology*, 8(23): 6677-6682.

Sukumar, K., Perich, M.J. and Boobar, L.R. (1991). Botanical derivatives in mosquito Diptera: Culicidae) control: A review. *Journal of the American Mosquito Control Association*, 7(2), 210-237.

Sukumar, K., Perich, M.J., and Boobar, L.R. (1991). Botanical derivatives in mosquito control: a review. *Journal of American Mosquito Control Association, 7,* 210-37.

Tare, V. and Sharma, R.N. (1991). Larvicidal activity of some tree oils and their common chemical constituents against mosquitoes. *Pesticide Research Journal, 3,* 169-172.

Tare, V., Deshpande, S., and Sharma, R.N., (2004). Susceptibility of two different strains of *Aedes aegypti* (Diptera: *Culicidae*) to plant oils. *Journal of Economic Entomology*, 97, 1734–1736.

Tolle, M. A. (2009) Mosquito-borne diseases; *Current Problem in Pediatric and Adolescent Health Care, 39* (4), 97–140.

Trease, G.E. and Evans, W.C. (1989). *Pharmacognosy* 13th (ed). ELBS/Bailliere Tindall, London. Pp. 345-6, 535-6, 772-3.

U.S.A., Environmental Protection Agency. (2007). *Mechanisms and mode of dioxin action*. http://www.epa.gov/ncea/pdfs/dioxin/nas-review/pdfs/part3

Vaisakh, M. N. and Pandey, A. (2012). The Invasive Weed with Healing Properties: A Review on *Chromolaena odorata*. *International Journal of Pharmaceutical Sciences and Research, Vol. 3(1): 80-83*

Valenzuela, J. G., Pham, V. M., Garfield, M. K., Francischetti, I. M. and Ribeiro, J. M. (2002). Towards a description of the sialome of the adult female mosquito *Aedes aegypti*. *Insect Biochemistry and Molecular Biology,* 32, (9), 1101–1122.

Valles, S. M., and Koehler, P. G. (1997) *Insecticides Used in the Urban Environment: Mode of Action*. From http://edis.ifas.ufl.edu

Veal, L., (1996). The potential effectiveness of essential oils as a treatment for headlice, *Complement therapy nursingand midwifery, 2,* 97–101.

Victor, B., Owoyele, J, Adediji, O. and Ayodele, Soladoye, O. (2005). Anti-inflammatory activity of aqueous leaf extract of *Chromolaena odorata. Inflammopharmacology, 13,* (6) 479–484

Vinayagan, A., Senthilkumar, N., and Umamaheswari, A. (2008). Larvicidal Activity of Some Medicinal Plant Extracts Against Malaria Vector *Anopheles stephensi. Research Journal of Parasitology, 3: 50-58.*

Wahid, I., Sunahara, T. and Mogi, M. (2003). Maxillae and mandibles of male mosquitoes and female autogenous mosquitoes (*Diptera: Culicidae*). *Journal of Medical Entomology,* 40 (2), 150–158.

Wang, W., Bostic, T.R., and Gu, L.W. (2010). Antioxidant capacities, procyanidins and pigments in avocados of different strains and cultivars. *Food Chemistry* 122(4), 1193–1198.

Ware, G.W. and Whitacre, D.M. (2004). *Introduction to Insecticides* (4th Edition). Willoughby: Meister Pro Information Resources.

Whitehorn, J. and Farrar, J., (2010). Dengue. *British. Medical Buletin,; 95*: 161–73.

WHO (2005). Guidelines for Laboratory and Field Testing of Mosquito Larvicides /CDS/WHOPES/GCDPP/.13. Geneva.

WHO (2003). HIV, Tuberculosis, Malaria and Neglected Tropical Diseases, Geneva.

WHO. (1973a). *Résistance des arthropodes aux insecticides.* (2nd Edition) Brown AWA and Pal R., (eds.). Série de Monographies, , *38*.

WHO. (1973b). *Specifications for pesticides used in public health* (4th edition). Geneva

WHO. (1993). *A global strategy for malaria control.* Geneva.

WHO. (1996). *Report of the WHO informal consultation on the evaluation on the testing of insecticides*, CTD/WHO PES/IC/96.1. Geneva.

WHO. (2012). *Malaria Report*. Geneva, Switzerland.

Wiwanitkit, V. (2010). Dengue fever: diagnosis and treatment. *Expert review of anti-infective therapy* 8 (7), 841–5.

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E. and Prior, R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agriculture and Food Chemistry*, 52(12), 4026–4037.

Yadav, R., Srivastava, .V.K., Chandra, R. and Singh, A. (2002). Larvicidal activity of Latex and stem bark of *Euphorbia tirucalli* plant on the mosquito *Culex quinquefasciatus*. *Journal of Communicable Diseases*, *34*, 264-9.

APPENDICES

APPENDIX 1.0

Serial Dilution Table

Stock Solution (%)	Stock solution (ppm)	Aliquot required (ml)	Final Conc. (ppm) in 100ml
1.0	10000.0	1.0	100
0.1	1000.0	0.5	50
0.01	100.0	0.25	25
0.001	10.0	1.0	100
0.0001	1.0	0.5	50
0.00001	0.1	0.25	25

APPENDIX 2.0

Column Elution Details For n-hexane extracts of *P. americana*

Void Volume of Column:

Length of column:

Diameter of column:

Length of silica in column: 28cm

Average Flow rate=2ml/min

Solvent gradients used n-hexane : ethyl acetate	Volume	Number of fraction
100% n-hexane	30ml each	1,2,3,4,5,
9.5:0.5	30ml each	6,7,8,9,10,
9:1	30ml each	11,12,13,14,15,
8:2	30ml each	16,17,18,19,20,21,22,23,
7:3	30ml each	24,25,26,27,28,29,30,31, 32,
6:4	30ml each	33,34,35,36,37,38,39,40,41,42, 43,
5:5	30ml each	44,45,46,

APPENDIX 3.0

TLC PROFILE POOLED FRACTIONS

Label	Fractions	Pooled fractions
nHPa1	1,2,3,4,5,6,7,8,9,10,11	F1-F11
nHPa2	12, 13, 14, 15	F12-F15
nHPa3	16, 17, 18	F16-F18
nHPa4	19, 20, 21,	F19-F21
nHPa5	22, 23, 24, 25, 26, 27, 28	F22-F28
nHPa6	29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41,	F29-F41
nHPa7	42, 43, 43, 44, 45, 46	F42-F46

APPENDIX 4.0

Column Elution Details For n-hexane extracts of *C. odorata*

Void Volume of Column:

Length of column:

Diameter of column:

Length of silica in column: 28cm

Average Flow rate= 1ml/min

Solvent gradients used n-hexane : ethyl acetate	Volume	Number of fractions
100% n-hexane	20ml each	1-29
9.8:0.2	20ml each	30-59
9:1	20ml each	60-68
8:2	20ml each	69-72
7:3	20ml each	73-120

APPENDIX 5.0

TLC PROFILE POOLED FRACTIONS

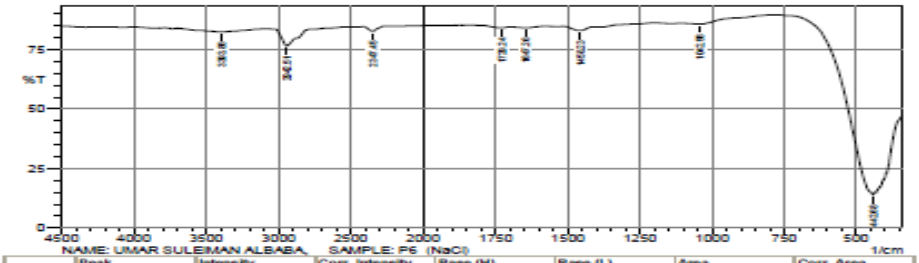
Label	Fractions	Pooled fractions
nHCo1	1,2,3,4,5,6,7,8,9,10,11	F1-F11
nHCo2	12, 13, 14, 15	F12-F14
nHCo3	16, 17,	F15-F17
nHCo4	19, 20, 21,22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36	F15-F36
nHCo5	37, 38, 39, 40,	F37-F40
nHCo6	41, 42, 43,	F41-F43
nHCo7	44, 45, 46, 47, 48, 49	F44-F49
nHCo8	50, 51, 52, 53, 54,	F50-F54
nHCo9	55, 56, 57, 58, 59, 60, 61,	F55-F61
nHCo10	62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72,	F62-F72
nHCo11	73, 74, 75, 76, 77, 78, 79, 80	F73-F80

APPENDIX 6.0

SHIMADZU

FTIR ANALYSIS RESULT NARICT,ZARIA

FTIR- 8400S FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER



NAME: UMAR SULEIMAN ALBABA, SAMPLE: P6 (NaCl)

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	442.68	13.979	1.163	453.29	434.96	15.283	0.268
2	1042.56	85.635	0.824	1082.1	917.18	10.167	0.227
3	1458.23	82.906	1.665	1504.53	1411.04	7.171	0.432
4	1647.26	84.084	0.037	1650.16	1638.58	0.87	0.001
5	1729.24	84.102	0.013	1731.17	1727.31	0.29	0
6	2347.45	82.659	2.004	2410.14	2281.61	11.408	0.674
7	2942.51	78.491	4.909	3024.48	2877.89	14.755	1.811
8	3393.86	82.39	0.003	3395.79	3373.61	1.865	0

Comment;
NAME: UMAR SULEIMAN ALBABA, SAMPLE: P6 (NaCl)

User; Administrator

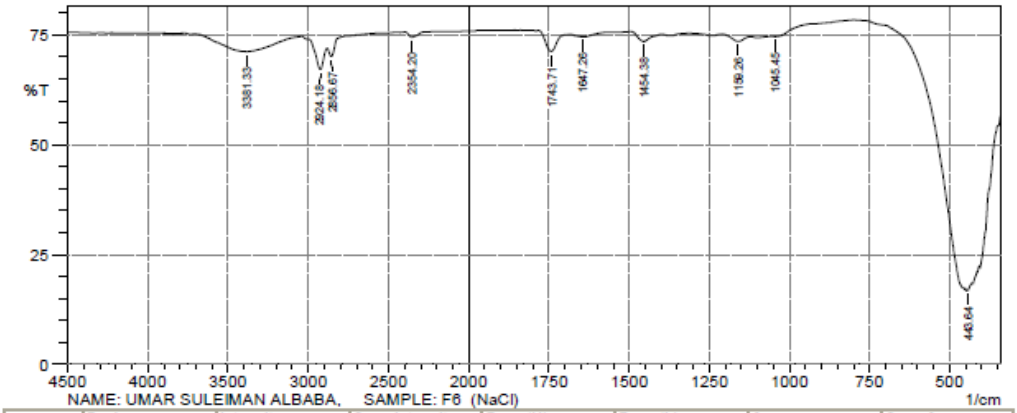
No. of Scans;
Date/Time; 5/30/2014 11:17:42 AM
Resolution;

APPENDIX 7.0

SHIMADZU

FTIR ANALYSIS RESULT NARICT,ZARIA

FTIR- 8400S FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER



NAME: UMAR SULEIMAN ALBABA, SAMPLE: F6 (NaCl)

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	443.64	16.852	0.16	444.61	431.1	10.174	0.016
2	1045.45	74.659	0.227	1057.99	800.49	29.188	-0.707
3	1159.26	73.553	1.299	1210.37	1121.64	11.444	0.303
4	1454.38	73.535	1.985	1494.88	1402.3	11.805	0.489
5	1647.26	74.591	0.045	1681.98	1645.33	4.619	0.006
6	1743.71	71.302	4.188	1783.25	1704.17	10.549	0.891
7	2354.2	74.5	1.227	2397.6	2220.14	21.841	0.435
8	2856.67	70.112	2.368	2881.75	2744.8	18.531	0.125
9	2924.18	67.168	5.545	2994.59	2881.75	17.183	1.732
10	3381.33	71.208	0.02	3383.26	3040.88	46.947	0.025