MOSQUITO-LARVICIDAL EFFICACY OF CRUDE AND FRACTIONS OF *PILOSTIGMA RETICULATUM* AND *COCUMA LONGA* AGAINST *CULEX QUINQUEFASCIATUS*, AN INTERMEDIATE HOST OF FILARIASIS

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ABSTRACT

The challenges posed by the management of mosquito vector by using synthetic insecticide have resulted into the development of resistance and have diverted the attention of mosquito control experts to the exploitation of plant products for mosquito vector control. This study was therefore designed to elucidate the mosquito larvicidal efficacy of the crude and solvent fractions of *Pilostigma reticulatum* and *Cocuma longa* against the filarial vector, *Culex quinquefasciatus*. The crude methanol extracts of the plants were assessed for the presence of bioactive photochemicals following standard procedure. Early 4th instar larve of the mosquito species were exposed to graded concentration (ranging from 0.2 to 1.00 mg/L) of the crude and solvent fractions of the two plant extract following the World Health Organization protocol for testing the efficacy of natural products. Mortality was measured after 24 hrs exposure period and lethal concentrations were evaluated using probit regression analysis. Phytochemical screening revealed the presence of flavonoids, tannis, saponin, alkaloids and terpenoid in both plant crude methanol extract. Saponin content (321.10±1.34 mg/100g) was significantly higher ($p<0.05$) than other phytochemical observed. The result also indicated that mosquito larvae mortality increases with increase in time of exposure and significantly ($p<0.05$) increase with increase in extract concentration. The n-hexane fraction recorded the highest larvae concentration compared to crude and ethyl-acetate fractions. Similarly, only the n-hexane recorded 100% larvae mortality compared to crude and fractions tested. The result showed that the *C. longa* n-hexane fraction was more potent against the tested mosquito species, with LC_{50} of 0.047 mg/L compared to the *P. reticulatum* n-hexane fraction (0.54 mg/L). Findings from the current study thus suggest the two tested plant as a promising candidate in the development of novel and effective larvicidal agents.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Mosquitoes are flying insects in the family Culicidae that are found around the world containing about 3500 species. The females of most mosquito species are hematophagy, sucking blood from other animals. This feature has made them the most deadly disease vector known, killing millions of people over thousands of years and continuing to kill more by the spread of the infectious diseases (Misganaw *et al*., 2019) chiefly in the sub-Saharah Africa. Several mosquito species belonging to genera *Anopheles*, *Culex* and *Aedes* are vectors of pathogens of various diseases including malaria, filiarisis, Japanese encephalitis (JE), dengue and dengue hemorrhagic fever, yellow fever among others (Becker *et al.,* 2003). Indeed, the present recrudescence of these diseases is attributed to high number of breeding places and the increasing resistance of mosquitoes to the current commercial insecticides (Chowdhury and Chandra, 2008).

In addition to personal protection and educating the public, the most successful method of minimizing the incidence of mosquito-borne diseases is to eradicate and control the mosquito vectors. This is performed principally by systematic treatment of the breeding places through a combination of environmental management and applications (Rozendaal, 1997). Despite centuries of efforts for most mosquito borne diseases, there are no effective methods by which to control the advance of epidemic in tropical regions (Rozendaal, 1997). Despite the immense resource presented by natural flora, control of mosquitoes still depends basically on the use of synthetic pesticides. They have become an essential component of insect pest management and in the past they have included mostly small molecular weight compounds, for example, organochlorines,

organophosphates, carbamates and others (Wood, 2003). The discovery of *Dichloro Diphenyl Trichloroethane's* (DDT) insecticidal properties in 1939 and the subsequent development of organochlorine and organophosphate insecticides suppressed natural product research since the answers to insect growth regulation were thought to have been found (Harzsch and Hafner, 2006). However, DDT ceased to be used due to insect resistance and environmental impact. Animals do not metabolize DDT very rapidly; instead it is deposited and stored in the fatty tissues and its biological half-life is about 8 years and if 2 ingested continues to build up at steady rate (Ndungu *et al.,* 2004).

In addition, the high cost of synthetic pyrethroids, environmental and food safety concerns, the unacceptability and toxicity of many organophosphate and organochlorine and the increasing insecticide resistance on a global scale have ruled out the miracle of chemical technology as a solution when it comes to containing the upsurge in mosquito population (Okon *et al.*, 2016). The synthetic larvicides are no doubt effective but have now been discarded due to deleterious effects on the ecosystem and non-target organisms. More concerted efforts have to go into studies to make environmentally friendly compounds viable for field use in vector control operations (Singh *et al.,* 2006). Therefore the search for an alternative vector control methods including biological control has attracted the attention of many researchers (Neetu *et al.,* 2007). An alternative for conventional chemical control is the utilization of natural products from fungi in the search for environmentally safe, biodegradable, low cost larvicides for vector control (Killeen *et al.*, 2002).

Despite its enormous historical successes, mosquito larval control remains a largely forgotten tool for mosquito control. With increasing interest in integrated vector management the potential of antilarval measures in Africa needs to be re-evaluated (WHO, 2005). The advantage of targeting the larval stage is that mosquitoes are killed

before they disperse to human habitations and that mosquito larvae, unlike adults, cannot change their behaviour to avoid control activities targeted at larval habitat (Chapman, 1974). Other alternative include introduction of larvivorous fishes into breeding sites to feed on mosquito larvae.

1.2 Statement of the Research Problem

During the past several decades, many synthetic organic insecticides have been developed and effectively used to eliminate mosquitoes. Unfortunately, the management of this disease vector by using synthetic insecticides has failed in part because the continuous and indiscriminate use of conventional chemical insecticides has resulted in the development of physiological resistance (Periswami *et al.,* 2015). In addition, there are long-term harmful effects on non-target organisms and the environment (Kwela *et al*., 2011). Besides, the complexities of the adult mosquito bio-ecology, as well as, biting behaviour have greatly hampered their effective management and control over the years. Experts have, therefore, identified the larval stage as an attractive target for insecticide development because they breed inwater (Nandita *et al*., 2008; Olayemi *et al.*, 2014).

1.3 Justification for the study

Natural products from plants are alternative sources of insect control agents since they contain a range of bioactive chemicals, which are selective and do not harm non-target organisms and the environment Plants have formed the basis of natural pesticides that make excellent leads for new pesticide development. During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic and chemical insecticides. However, more concerted efforts

have to go into these studies to make these environment friendly compounds viable for field use and for large-scale vector control operations (Raveen *et al.*, 2014).

1.4 Aim and Objectives of the Study

The aim of the study was to evaluate the bio-pesticidepotency of the crude and fractionated extract of *Pilostigmareticulatum* and *Cocuma longa* against 4th instar larvae of *Culexquinquefasciatus* while the objectives are;

Objectives

The objectives of this study were to determine:

i. Qualitative and quantitative phytochemical constituents of the crude methanol extract of *Cocuma longa* and *Pilostigma reticulatum*

ii. larvicidal activity of crude methanol extracts of *Cocuma longa* and *Pilostigmareticulatum*

iii. larvicidal activity solvent fractions of *Cocuma longa* and *Pilostigma reticulatum*

iv. lethal concentration of the extract of *Cocuma longa* and *Pilostigma reticulatum*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mosquito Vectors

Mosquito are vectors of the world most deleterious diseases. These diseases are comprehensively conveyed to individuals by bites from infected female mosquito (Ghosh *et al.,* 2012). Most species of mosquitoes are harmless. However, several species carry and transmit diseases. Mosquitoes are the only agents that carry and transmit malaria, yellow fever, dengue fever, and filariasis to man. They are the leading agents in transmitting several forms of viral encephalitis. Mosquitoes also transmit certain diseases to animals.

Furthermore, even among mosquitoes that do carry important diseases, neither all species of mosquitoes, nor all strains of a given species transmit the same kinds of diseases, nor do they all transmit the diseases under the same circumstances; their habits differ. For example, some species attack peoples in houses, and others prefer to attack people walking in forests. Accordingly, in managing public health, knowing which species, even which strains, of mosquitoes with which one is dealing is important (Lawal *et al.,* 2016). According to the world health organization (WHO), about half of the world population (3.3 billion) is at risk of mosquito borne diseases. Although the past several years have witnessed tremendous increase in control measures of mosquito bore diseases. In Nigeria for instance, the entire population of over 170 million is at risk of malaria which is responsible for about 60% and 30% of outpatients' visits and hospitalizations respectively (Positive Material Identification, Hill *et al.*, 2009). Insecticide-based control measures such as Long Lasting Insecticide treated mosquito Nets (LLINs) and Indoor Residual Spraying (IRS) are widely used to control malaria

vector. These measures are currently inadequate (Okorie *et al.,* 2011) and not sufficient to halt mosquito borne disease transmission and would likely contribute to the eventual emergence of insecticide resistant mosquitoes (Ramson *et al.,* 2009; Hunt *et al.,* 2011).

2.2 Distributions and Occurrence of Mosquitoes

Mosquitoes have a worldwide distribution, occurring in all regions of the world except for Antarctica (Olayemi *et al.,* 2014). In warm and humid tropical regions, they are active for the entire year, but in temperate regions, they hibernate over winter. Arctic mosquitoes may be active for only a few weeks. The larval head is short and stout becoming darker towards the base. The mouth brushes have long yellow filaments that are used for filtering organic materials. The abdomen consists of eight segments, the siphon, and the saddle. The siphon is four times longer than it is wide with multiple setae tuffs. The saddle is barrel shaped and located on the ventral anal papillae protruding from the posterior end (Okon *et al.*, 2016).

2.3 Life Cycle of Mosquitoes

Life cycle of mosquitoes takes place in four distinct stages, from egg stage through to adult stage. According to Centers for Disease Control and Prevention (CDC) the entire life cycle of mosquitoes lasts approximately 8-10 days at room temperature, depending on the level of their feeding. There is an aquatic phase (exhibited by the eggs, larvae and pupae) and a terrestrial phase (exhibited by the adults) in the life-cycle. Eggs: Female mosquitoes lay eggs about every third day during their lifespan, usually in clumps of 100 to 300 eggs. The eggs are deposited either as “rafts” or singly (based on genera) floating on the surface of standing water and or on the ground in areas that regularly flood. Mosquitoes can lay eggs in as little as one inch of water. The eggs, generally

white and hardy when laid, cannot hatch unless they are in water, usually for two to three days.

Eggs of *Aedes* and *Culex* can survive desiccation for months and hatch once submerged in water (American Mosquito Control Association, 2014). Larvae: When the eggs hatch, the larvae emerge. The mosquito larva has a well developed head with mouth brushes used for feeding, a large thorax with no legs, and a segmented abdomen.They are called "wigglers" because of their mode of swimming. The 9 larvae of most mosquitoes obtain oxygen from the atmosphere by coming to the water surface. Most of the time, they hang from the surface of the water, breathing through tubes (siphons). The wigglers (larvae) pass through four instar stages before pupae emerge. Majority of larvae feed on suspended particulate and organic matter in the water and microorganisms which they extract from the water with filamentous mouth brushes. Some larvae resort to scavenging or cannibalism when food is scarce.

Pupae: The pupae are called "tumblers" because of the way they fall into the deepest part of the water when threatened by predators. They are comma-shaped, partially encased in cocoons, with the head at one end and tiny flippers at the other. The pupae do not feed while developing, but breathe through tubes (siphons) like the larvae. It takes about four days for the adult mosquito to emerge out of it.

Adults: The newly emerged adults climb out of the water to rest and wait for their bodies to dry out. The males will take a day or two to fully develop their reproductive organs, and then seek out a female, by the sound of her wingbeats, for mating. They will live about three to five days after that, feed on fruit and plant nectar. The females mate once, but continue laying eggs after every blood meal. Under the best conditions, they can live up to a month or two (American Mosquito Control Association, 2014).

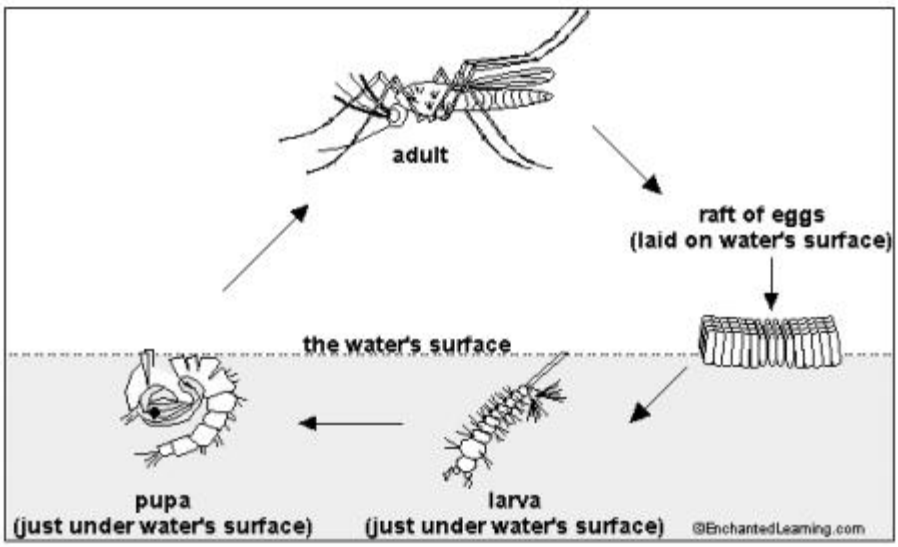

Figure 2.1: Diagram of a generalized life cycle of mosquito (Source: www.enchantedlearning.com)

2.4 Taxonomy of *Culex* Mosquito

Kingdom: Animalia; Phylum: Arthropoda; Class: Insecta; Order: Diptera; Suborder: Nematocera; Infraorder: Culicomorpha; Superfamily: Culicoidea; Family: Culicidae; Subfamilies: Anophelinae, Culicinae and Toxorhynchitinae. Mosquitoes belongs to the family Culicidae, order Diptera (the two-winged flies). The family is a large and abundant group which occurs throughout temperate and tropical regions of the world, and well beyond the Arctic Circle. The family includes 3,540 species, classified in three subfamilies and 112 genera. The subfamily Anophelinae has three genera and Culicinae has 109 genera. Several species belonging to genera Aedes, Anopheles and Culex are vectors. The most common, and most dangerous, are the various species in the Culex, Anopheles, and Aedes genera (Tennyson *et al.*, 2012).

Culex is a genus of mosquitoes, consists of several species of which serve as vectors of important human diseases, such as West Nile virus, filariasis, japanese encephalitis and avian malaria (Syed *et al.*, 2009). The adult can measure up to 4-10mm (0.16-

0.39inches) and mophologyically have three body regions regular to insect which entails: head, thorax and abdomen. As a fly (Diptera), it has a pair of wings (Hill *et al.,* 2009).

2.5 Scientific Classification of *Culex quinquefasciatus*

Kingdom- Animalia; phylum- Arthropoda; class- insecta; order- Diptera; Family- culicidae; sub-family- culicinae; genue- *Culexquinquefasciastus* Mosquitoes control.

2.6 Life cycle of Mosquito

There are four distinct stages of mosquito**;** Egg, larva, pupa and adult. Each stage has a unique appearance. Understanding the mosquito life cycle is vital for valuable vector control and disease obstacle strategies. Mosquitoes have a complex life cycle.

2.6.1 Distribution of Mosquitoes

Culex quinquefasciatus is a domesticated species which is often found living in close proximity to humans (Derraik and Slaney, 2005). Their larvae are found in freshwater habitats such as pools, ditches, ponds, and even in effluents of sewage treatment plants (Derraik and Slaney, 2005). These mosquitoes tend to hibernate over the winter and breed during the warmer months, laying rafts of eggs at night on the surface of standing water anywhere it can be found. Over a period of about two weeks, the eggs hatch, larvae emerge, develop into pupae, and then into adult mosquitoes. They normally do not travel more than one kilometer from a hatching point (Derraik and Slaney, 2005). Adults feed primarily from dusk until a few hours after dark (are most active at dusk, active daytime biters) and are considered aggressive and persistent biters, although they prefer birds to people. Females are anautogenous; need the protein in blood to develop eggs, before laying the first batch of eggs (Oda *et al*., 2002). They can live up to a month. Aedes aegypti immature stages are found in temporary floodwater pools, fresh and brackish marshes containers. Adults are abundant and bite readily outdoors at all

hours of the day so it is not uncommon for them to enter homes to feed on human blood (Public Health Pest Control, 2001). Adult mosquito in this genus prefers to breed in tree holes, overflow ditches, and old discarded tyres. As a predominantly tropical and subtropical group, Aedes mosquitoes tend to breed in warm weather, although some species can survive in colder environments. The adults feed day and night, and several of the species 16 are considered particularly troublesome. Its lifespan range from two weeks to a month, depending on environmental conditions (Maricopa, 2006).

Aedes aegypti survives in three polytypic forms; domestic, sylvan and peridomestic forms. The domestic form breeds in urban areas, often around or inside houses. The sylvan form breeds in tree holes, generally in forest and the peridomestic form thrives in environmentally modified areas such as coconut groves and farms (Foster and Walker, 2002). Aedesvittatus (Bigot) immature stages prefer to breed in rock pools, coconut shell, latex collecting containers, mud pot, plastic container, tank and sometimes treehole/ tree stump (Jomon *et al.,* 2009). Their eggs can survive drying (desiccation) and hatch once flooded by water in the natural rock hollows. When rains occur, the rock hollows fill with water and the submerged eggs are stimulated to hatch. Thus, in countries with a distinct dry season, the eggs must be resistant to prolonged dehydration, while awaiting the rains. This ability is common among Aedes species (Derek, 2004). Aedesvittatus is the dominant species of mosquito that breeds in rock pools. Research shows that the species is catholic in its choice of breeding microhabitat in rock pools and the least affected by physicochemical conditions of the rock pools (Adebote *et al*., 2008).

2.6.2 Responses of mosquito to insecticides

The capacity of mosquito for disease transmission is greatly influenced by local environmental factors that conditioned their vectorial fitness, usually manifested in wing symmetry. Degree of wing symmetry in mosquito, on the other hand reflect how well-formed internal organs and physiological processes are and thus their vectorial competence, i.e. ability to support pathogen development (Muturi *et al*., 2012). Also, adult body size of mosquito is largely dependent on larva dietary condition, with respect to quality and quantity of nutritional reserves acquired from breeding habitat. Such nutritional reserves are used for egg formation thus, translating into fecundity and by extensions adult population density. If insufficient nutritional reserves are acquired population among surviving larvae may be delayed for as much as three weeks, even the emerging of larvae and adult mosquitoes tend to be smaller than the threshold required for significant vectorial capacity. This is so, because in mosquitoes, newly emerged adult go through a teneral phase within 24 hour post-emergence, during which convert anatomical, physiological and behavioural maturation taken place, the quality of which are dictated by the quantity of teneral reserves (Olayemi *et al.,* 2014).

2.7 Public Health Importance of Mosquito

Mosquitoes can be annoying, causing serious problems in human domain. They interfere with human work and spoil hours of human leisure time. They can attack farm animals which result in loss of weight and decreased production. Some mosquitoes are capable of transmitting diseases such as malaria, yellow fever, dengue, filariasis and encephalitis to humans and animals (American Mosquito Control Association, 2014).

Culex quinquefasciatus is vector of many pathogens of humans, as well as both domestic and wild animals. Viruses transmitted by this species include West Nile virus, St. Louis encephalitis and Western equine encephalitis viruses. The species is the principal vector of St. Louis encephalitis in the southern U.S.A. Although *Culex*

quinquefasciatus is not considered the likely primary vector of West Nile virus in Florida, it likely plays an important role in maintaining the virus within bird populations, and is capable of transmitting it to humans (Hill and Connelly, 2013). Certain species of Aedes are also important in the transmission of yellow fever and dengue, for example *Aedes albopictus*, (the Asian tiger mosquito), transmits dengue fever and eastern equine encephalitis, while *Aedes aegypti* (the yellow fever mosquito) transmits dengue and yellow fever also. *Aedes aegypti* bites cause minor localized itching and irritation to the human skin, this makes outdoor adventure very unpleasant (Foster and Walker, 2002). Due to their small size and the limited probing abilities of the proboscis, mosquitoes are limited to feeding on nectar sources within flowers that have shallow or flat corollas. Unlike the relatively large pollinators like bees and butterflies, mosquitoes can feed on nectar efficiently without coming into contact with pollen-coated stamens. Thus, although they may transfer some pollen during the course of acquiring meal of nectar, mosquitoes are not important pollinators in general. Mosquitoes are natural component of many aquatic and terrestrial ecosystems. Like other aquatic insects with terrestrial adult stages, mosquitoes provide a link between aquatic and terrestrial habitats.

2.8 Phytochemicals

Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources (Shahi *et al.,* 2010). Botanicals are basically secondary metabolites that serve as a means of defence mechanism of the plants to withstand the continuous 18 selection pressure from predators and other environmental factors. Insecticidal effects of plant extracts vary not only according to plant species, mosquito species, geographical varieties and parts used, but also due to extraction methodology adopted and the polarity of the solvents used during extraction (Ghosh *et al*., 2012). A wide selection of plants from herbs, shrubs and large trees are used for extraction of mosquito toxins.

Phytochemicals can be extracted either from the whole body of herbs, shrubs or from various parts like fruits, leaves, stems, barks, roots and or seeds of larger plants. In all cases where the most toxic substances were concentrated upon, are used for mosquito control (Ghosh *et al.,* 2012). 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). At present, phytochemicals make up to one percent of worlds pesticide market (Isman, 1997). The efficacy of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant parts used, age of plant parts (young, mature or senescent), solvent used during extraction as well as upon the available vector species (Ghosh *et al.,* 2012). (Sukumar *et al.,* 1991) described the existence of variations in the level of effectiveness of phytochemical compounds on target mosquito species vis-à-vis plant parts from which these were extracted, responses in species and their developmental stages against the specified extract, solvent of extraction, geographical origin of the plant, photosensitivity of some of the compounds in the extract, effect on growth and reproduction. It has been shown that the extraction of active biochemical compounds from plants depends upon the polarity of the solvents used. Polar solvent will extract polar molecules and non-polar solvents extract non-polar molecules. This was achieved by using solvent 19 systems ranging from petroleum ether, the most nonpolar (polarity index of 0.1 that mainly extracts essential oil) to that of water, the most polar (polarity index of 10.2) that extracts biochemical with higher molecular weights such as proteins and glycans (Ghosh *et al.,* 2012).

2.9 Strategy for Mosquito Control

2.9.1 Source reduction

Open water marsh management (OWMM) involves the use of shallow ditches, to create a network of water flow within marshes and to connect the marsh to a pond or canal. The network of ditches drains the mosquito habitat and lets in fish, which will feed on mosquito larvae. This reduces the need for other control methods such as pesticides. Simply giving the predator's access to the mosquito larvae can result in long-term mosquito control (Chevillon *et al*., 1999).

2.9.2 Experimental biocontrol methods

In 2014 and 2018, research was reported into other genetic methods including cytoplasmic incompatibility, chromosomal translocations, sex distortion and gene replacement. Although several years away from the field trial stage, if successful these other methods have the potential to be cheaper and to eradicate the *Culex* mosquito more efficiently.

2.9.3 Trap larva

This is a process of achieving sustainable mosquito control in an eco-friendly manner by providing artificial breeding grounds with an ovitrap or an ovillanta utilizing common household utensils and destroying larvae by non-hazardous natural means such as throwing them in dry places or feeding them to larvae eating fishes like *Gambusiaaffinis*, or suffocating them by spreading a thin plastic sheet over the entire water surface to block atmospheric air.

2.9.4 Trap adult

In several experiments, researchers utilized mosquito traps. This process allowed both the opportunity to determine which mosquitoes were affected, and provided a group to be released with genetic modifications resulting in the OX513A variant to reduce reproduction. Adult mosquitoes are attracted inside the trap where they die of dehydration.

2.9.5 Use of DDT

DDT was formerly use throughout the world for large area mosquito control, but it is now banned in most developed countries. DDT remains in common use in many developing countries (14 countries were reported to be using it in 2009) which claim that the public health cost of switching to other control methods would exceed the harm caused by using DDT.

2.10 Medicinal use of *Pilostigma reticulatum*

The plant, *Pilostigma reticulatum,* is used widely in Africa as a traditional medicine for the treatment of many diseases, such as malaria, tuberculosis, and diarrhea.

(Dosso *et al.,* 2012). The leaves are use against fever and as a tranquillizer, and for the treatment of a range of ailments including colds, bronchitis, headache, rheumatism, ophthalmic, toothache, mumps, syphilis, vertigo and epilepsy. Leaf preparations are often applied on wounds, ulcers and sores; they are considered haemostatic, antiseptic and cicatrising. Ground fresh leaves are applied in case of inflammation. Young leaves are eaten raw against nausea. Boiled leaves are rubbed in against lumbago. In northern Senegal a decoction of the leaves in a vapour-bath is used against conjunctivitis. Leaf decoctions are taken by women in labour to ease delivery, and are used in draught and in baths as a sedative and against epilepsy and possession. Leaf decoctions are also taken against dysentery, haemorrhoids, malaria and hernia. In Nigeria leaf decoctions

are used to foment fractures and to get rid of guinea worm. A leaf decoction is rubbed into scarifications for the treatment of leg pain. Leaf infusions are used in drinks or baths as a sedative and anti-rachitic for new-born children, and to stimulate their appetite. Macerations of young leaves and flower buds are given against rickets in babies, kwashiorkor and anorexia.

2.10.1 Prospects of *Pilostigma reticulatum*

Pilostigma reticulatum is a valuable multipurpose plant, yielding a wide range of useful products. In Burkina Faso, for instance, it is becoming more and more important, because of the decline of other traditional agroforestry species. Integration of *Pilostigma reticulatum* in the traditional agroforestry systems in semi-arid and arid countries is important for sustainable use of the species. Further research on the domestication potential of the species is worthwhile. The antimicrobial and anti-inflammatory properties warrant further research for pharmaceutical uses. The leaf extract from the plant was found to exhibit anti-microbial activity against some bacteria and fungi such as *Staphylococcus aureus* (Sidiki, 2013).

2.10.2 Description of *Pilostigmareticulatum*

Dioecious shrub or small tree up to 10–15m tall bole short, rarely straight, up to 30 cm in diameter; outer bark deeply fissured to cracked, grey to brown, inner bark pink to red; crown rounded and dense; branches grey, waxy and glabrous. Leaves alternate, conspicuously bi-lobed; petiole 1–3.5 cm long, swollen at both ends; blade 5–12 cm × 4–18 cm, cordate or rounded at base, lobes rounded or more or less cuneate, coriaceous, globous, greyish-green, palmately veined with 8–11 basal veins. Inflorescence an axillary or terminal panicle, 5–15 cm long, shortly pubescent. Flowers unisexual, c. 2.5 cm in diameter; calyx 5-toothed, 15–20 mm long; petals 5, obovate, white with pink

stripes; male flowers with 10 stamens, anthers brown. Fruit an oblong pod 15–30 cm × 2.5–5 cm, straight, undulate or twisted, woody, hard, glabrous or sparsely pubescent, brown, flat, pruinose, sometimes twisted and cracked, indehiscent and persisting, many-seeded. Seedling with epigeal germination.

2.10.3 Other botanical information

*Pilostigm*a comprises 3 species in tropical Africa, Asia and Australia. *Pilostigma reticulatum* is frequently confused with *Piliostigma thonningii* due to similarity in appearance, but the latter has larger leaves with hairs on the lower surface, and usually occurs in less dry areas.

2.10.4 Growth and development

The growth of *Pilostigma reticulatum* is recorded to be slow. Flowering is in the dry season. In Benin flowering and fruiting occur in October. In drier areas *Pilostigma reticulatum* is semi-deciduous, losing most of its leaves at the end of the dry season, but in more humid zones the leaves are often persistent. The plant resprouts after the stem has been cut. Animals eating the fruits contribute to dispersal of the seeds (Sidiki, 2013).

2.11 Ecology

Pilostigma reticulatum occurs from sea level up to 2000 m altitude in areas with an annual rainfall of 200–400–1000 mm, mainly on heavy and poorly drained soils, but also on sandy soils. It is a pioneer species in woodland, wooded scrubland, wooded grassland, valleys and disturbed habitats such as cultivated fields, fallows and roadsides. The species is common and locally abundant. The *Pilostigma reticulatum* plant is used widely in Africa as a traditional medicine for the treatment of many diseases, such as malaria, tuberculosis, and diarrhoea (Dosso*etal.,* 2012) .The leaf extract from the plant

was found to exhibit anti-microbial activity against some bacteria and fungi such as Staphylococcus aureus (Sidiki, 2013). The Figure 1 below showed the diagrammatic representation of *Pilostigma reticulatum*.

Figure 2.4 *Pilostigmareticulatum* plant commonly known as camel foot (Dosso*et al.,* 2012).

2.12 Morphology (description)

The plant *Pilostigma reticulatum* (Leguminocaea, Fabaceae) is woody plant or tree of 4 – 15m in height with a rounded crown and a short but often crooked bole. The twigs are hairy. The bark is rough and longitudinally fissured, being creamy brown later. Leathery leaves up to 15 x 17 cm, bi - lobed one eight to one third the way down with a small bristle notch, glossy above and heavily veined and somewhat rusty hair below. Flowers with five white to pink petals, pendulous, unisexual with male and female usually on separate trees, ovary topped by a thick flattened globose stigma, pods are indehiscent, up to 26 x 7 cm with rusty brown hairs which wear off as the pods mature, becoming somewhat concorted as they age. The pods persist on the tree but finally fall and decay on the ground to pea sized seeds. An edible pulp surrounds these seeds. This species roots deeply this help them to resist or survive strong winds and also to get water in time of drought. The generic epithet *Pilostigma* have cap like stigma. *Pilostigma reticulatum* is 18 found commonly in open woodland and wooded grasslands of sub humid Africa at medium to low altitudes (Akerele, 1991).

Morphologically *Pilostigma reticulatum* is a woody plant found in open woodland and wooded grasslands of sub humid Africa. It is found throughout Africa except in Somalia and is always associated with Annona senegalensis, *Grewiamollis* and *Combretum spp*.

2.12.1 Ethno-medicinal uses

Ethno-medicinal (Traditional) uses of *Pilostigma reticulatum* and other species in the genus *Pilostigma* and other species in the genus have been reported to have a wide range of uses to mankind ranging from food for man and animals and also a wide range of medicinal uses (Ibewuike *et al*., 1996). The medicinal uses include treating loose stool in teething children, wound dressing, ulcers treatment, worms' infestation, arrest bleeding, inflammations, bacterial infections, gonorrhoea, stomach ache, headache, etc (Ozolua *et al*., 2009).

The roots and twigs have been used locally in the treatment of dysentery, fever, respiratory ailments, snake bites, hookworm and skin infections and the leaf extracts has been used for the treatment of malaria all over Eastern Nigeria (Kwaji *et al*., 2010). The plant is used in ropes making, making of dyestuff or tanning of leather, household utensils, roofing ties, fencing, bridge building and farm implements, because they are deep rooted they are used as erosion control measures, the woods are used as stakes to support plants of weak stems or creepers like yams.

Figure 2.5 describes *Cucuma longa*. L. (syn. *C. domestica*. Vahl.) is a perennial rhizomatous herb of the family Zingiberaceae. The rhizome is the source of turmeric, which has use as a condiment and coloring agent in medicines, confectionery and curry powder. Turmeric has a long traditional use in the Chinese and Ajurvedic systems of medicine, particularly as an anti-inflammatory agent, and for the treatment of flatulence, jaundice, and menstrual. Constituents of the leaf, rhizome, and flowers have been studied extensively. The rhizome essential oil is reported to exhibit antimicrobial insecticidal, larvicidal, repellency, and antioxidant activities (Nelson *et al.,* 2017). The leaf oil has also been reported to exhibit fumigant toxicity against stored-products beetles. This paper reports the chemical constitution of the essential oils of the leaf and rhizome of *C. longa*. and their toxicity to the larvae of *Anopheles gambiae*. Mosquitoes (Sacchetti, 2005).

Figure 2.5: *Curcuma longa*

2.13 Medicinal use

Powdered rhizome is used to treat wounds, bruises, inflamed joints and sprains in Nepal. In current traditional Indian medicine, it is used for the treatment of biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis (Jain and DeFillips, 1991). A short clinical trial in 18 patients with definite rheumatoid arthritis showed significant improvement in morning stiffness and joint swelling after two weeks of therapy with oral doses of 120 mg/ day. Application of the powder in combination with other plant products is also reported for purification of blood and for menstrual and abdominal problems.

In patients undergoing surgery, oral application of curcumin reduces post-operative inflammation. Recently, curcumin has been formulated as slow-release biodegradable microspheres for the treatment of inflammation in arthritic rats. It is evident from the study that curcumin biodegradable microspheres could be successfully employed for therapeutic management of inflammation (Kumar *et al*., 2002).

Turmeric is a member of the Ginger family and is one of the most valuable holistic ingredients you can feed to your poultry to help prevent disease in your flocks. Turmeric is responsible for the golden colour of our feeds.

Turmeric's hepatoprotective effects, evidenced in a number of animal studies, suggest it may be used in cases of toxic insult due to exogenous toxins from lifestyle and environmental exposures. Curcumin has choleretic activity that increases bile output and solubility, which may be helpful in treating gallstones (Park *et al.,* 2000).

Curcumin is a potent anti-inflammatory with specific lipoxygenase- and COX-2-inhibiting properties. Animal, *in vitro* and *in vivo* studies demonstrate turmeric's

effectiveness at decreasing both acute and chronic inflammation. A double-blind, crossover, placebo-controlled human study of 42 patients with osteoarthritis used a combination product containing turmeric, *Boswellia serrata*, *Withania somnifera* and zinc. After three months on the combination or placebo, patients noted a significant reduction in pain ($p<0.001$) and disability ($p<0.05$).

Numerous animal, *in vitro* and *in vivo* studies have demonstrated the anticarcinogenic effects of turmeric and its flavonoid component curcumin against colon, breast and prostate cancers, as well as melanoma (Duvoix *et al.,* 2005). Curcumin, one of the most studied chemopreventive agents that allow suppression, retardation or inversion of carcinogenesis.

Curcumin is also described as an anti-tumoral, anti-oxidant and anti-inflammatory agent capable of inducing apoptosis in numerous cellular systems (Duvoix *et al.,* 2005).

Zhang *et al.,* investigated that the mechanism of anti-tumor effects of curcumin on human lung cancer cell (A549). Curcumin can interfere with cell growth cycle of A549 cell and suppress cell growth.

Curcumin can suppress the growth; induce apoptosis of bladder cancer EJ cell in vitro. Its mechanism is related with down-regulations of the expressions of NF-kappa B and Cyclin D1. Curcumin has great potential for the treatment of bladder cancer (Sun *et al.,* 2004).

Curcumin inhibits the proliferation in both estrogen receptor (ER) positive MCF-7 cells and ER negative MDA-MB-231 cells. Means Curcumin exerts multiple suppressive effects on breast carcinoma cells (Di *et al*., 2003).

Curcumin induced melanoma cell apoptosis and cell cycle arrest, curcumin arrested cell growth at the G(2)/M phase and induced apoptosis in human melanoma cells by inhibiting nuclear factor (NF) kappa B activation and thus depletion of endogenous nitric oxide. Therefore, Curcumin should be considered further as a potential therapy for patients with melanoma (Zheng *et al.,* 2004).

Animal and *in vitro* studies have shown the potential for turmeric to decrease blood lipids. Further clinical studies need to be performed in this area to discover optimal dosages for cardiovascular protection and lipid lowering.

An open, phase II trial was performed on 25 patients with endoscopically-diagnosed gastric ulcer. Participants were given 600 mg powdered turmeric five times daily. After four weeks, ulcers had completely healed in 48 percent of patients. The success rate increased over time, with 76 percent being ulcer free after 12 weeks of treatment. No significant adverse reactions or blood abnormalities were noted.

Thirty-two patients with chronic anterior uveitis (inflammation of uvia layer) took 375 mg curcumin three times daily for 12 weeks. Curcumin was effective in 86 percent of individuals, and was as effective as corticosteroid therapy, the only available standard treatment.

Curcuma longa rhizome extract showed blood glucose lowering activity in experimental, induced- diabetic rats. Curcumin treatment also significantly reduced macrophage infiltration of white adipose tissue, increased adipose tissue adiponectin production, and decreased hepatic nuclear factor-kappa B activity, hepatomegaly, and markers of hepatic inflammation. We therefore conclude that orally ingested curcumin reverses many of the inflammatory and metabolic derangements associated with obesity and improve glycemic control in case of diabetic rat (Weisberg *et al.*, 2008).

Curcumin shows anticoagulant activity by inhibiting collagen and adrenaline-induced platelet aggregation *in vitro* as well as *in vivo* in rat thoracic aorta.

Petroleum ether and aqueous extracts of turmeric rhizomes show 100% antifertility effect in rats when fed orally. Implantation is completely inhibited by these extracts. Curcumin inhibits 5*a*-reductase, which converts testosterone to 5*a*-dihydrotestosterone, thereby inhibiting the growth of flank organs in hamster. Curcumin also inhibits human sperm motility and has the potential for the development of a novel intravaginal contraceptive.

Alzheimer's disease (AD) involves amyloid (Abeta) accumulation, oxidative damage and inflammation. The phenolic yellow curry pigment curcumin has potent anti-inflammatory and antioxidant activities and can suppress oxidative damage, inflammation, cognitive deficits, and amyloid accumulation.

The cytokine macrophage migration inhibitory factor (MIF) has recently emerged as a crucial factor in the pathogenesis of rheumatoid arthritis (RA). Curcumin and caffeic acid were found to be the most potent inhibitors (Molnar and Garaj, 2005).

Cucuma longa is widely cultivated for its rhizomes and rhizome powder also commonly known as turmeric and turmeric powder which is used as a bright yellow-orange culinary spice. It has been known as poor man's saffron because it offers a less expensive alternative yellow colouring.

In *Cucuma longa*, curcumin is the primary pigment and is generally used in various food industries as a food color. It is mainly used in dairy products, beverages, cereal, confectionary, ice cream, bakery, and savory products. Turmeric is mostly used in flavored milk drinks, cultured milk and desserts to obtain lemon and banana colors in

dairy. Turmeric is added at higher levels to sausages, pickles, relishes, sauces, dry mixes, and fish due to its original usage as a spice.

Cucuma longa L. (turmeric) (Zingiberaceae) has been known for centuries not only as one of the dietary spice plants of South-East Asia. Rhizome of this plant has also been used as a safe remedy against many ailments (mostly DM) in many countries, principally in China and India. Recent investigations (2016), revealed hypolipidemic properties of ethanolic extract of turmeric which can be used in the treatment of hyperlipidemia. According to the authors India is the diabetic capital and leads the world with largest number of diabetic patients about 40.9 million in the year 2007 and probably to 69.9 million by the year 2020.The active substance of turmeric forms yellow orange powder (curcuminoids) with the main constituent curcumin (77%) and its natural derivatives: demethoxy curcumin (DMC) (17%) and bisdemethoxy curcumin (BDMC) (6%) (Ravindranath and Chandrasekhara, 2010).

In recent years, curcumin has gained great scientific attention because of its wide range of important pharmacological properties, including anti-inflammatory, antioxidant, antidiabetic, antiangiogenic, anti-mutagenic, anti-infective and anticancer activities. In the literature there are many publications describing the beneficial role of curcumin in the prevention and treatment of different chronic ailments (such as diabetes type 2, Alzheimerís disease, multiple sclerosis, atherosclerosis, great variety of cancers) as well as in the improvement of its bioavailability in many organ disorders.

Curcumin is a natural substance inhibiting proinflammatory markers, which is highly safe, non-toxic (even in 8 g pro die dose), easily available, and side effect-free (20). The chemical structure of curcumin was established over 100 years ago by a group of Polish researchers who also confirmed it later by total synthesis (9, 19). A lot of detailed

investigations are connected with biomedical turmeric application into the treatment of diabetes and diabetes-related complications and also with the elaboration of active nanotechnological formulations of this natural product in order to enhance its solubility and bioavailability. The researchers and practitioners are concerned about difficulties to use curcumin beneficial properties in practice. In this context several formulations have been proposed, such as the encapsulation of curcumin in liposomes or in polymeric micelles, complex formations with cyclodextrin, polymer-curcumin conjugates and others to ameliorate its physical and biological properties. A parallel increase in liposomal curcumin solubility and its activity is observed. This present review shortly synopsizes results from several latest reviews and the investigation data (from June 2015 till February 2016), indicating the opportunities of application of curcumin in the traditional medicine in spite of its adverse physicochemical properties (mainly of hydrophobic nature), biological instability (poor absorption, rapid metabolism in the gastro-intestinal tract) and consequently limited bioavailability.

2.14 Physico-chemical Properties of Curcumin

Physico-chemical properties of Curcumin ñ yellow-orange substance, chemical formula C21H20O6, 1.7-bis-(4-hydroxy-3- methoxyphenyl)-hepta-1.6-diene-3.5-dione; m.w. 368.39 g/mol, m. p. 187-188OC, poorly soluble in water (0.6 µg/mL) and most organic solvents, appears in nature in two tautomeric: keto and enol forms; the enol form is more stable in the solid state and in solution. Maximum absorption (λmax in methanol) at 430 nm.

Extraction of curcumin from its natural source (Curcuma elongaerhizoma) involves neither difficulties nor significant costs. Its main obstacle in conducting both clinical and chemical studies is in the opposition to other TNF inhibitors and its low

bioavailability. Numerous research reported that curcumin is poorly absorbed from gastrointestinal tract, metabolized (via glucuronidation and sulfation) and eliminated rapidly. Only the small amount of curcumin is distributed from blood to tissue. Mostly, the concentration level of curcumin in tissues remains under the detection limit. Therefore, numerous formulations were created in order to improve bioavailability of this compound. Although nanotechnology gives an opportunity to increase hydrophobic drugs absorption, little research was conducted on curcumin nanoparticles (20). Liposomes can carry hydrophobic and lipophobic particles due to its amphiphilic properties. Liposome-encapsulated curcumin is now being developed to be introduced into clinical trials as it has a more potent growth-inhibitory effect on colorectal cancer in in vivo trial than oxaliplatin. Another approach to improve curcumin absorption includes the modification of its chemical structure to create analogues with more favorable properties. Curcuminoids The name curcuminoids refers both to the natural diferuloylmetane derivatives (curcumin, DMC, BDMC) and synthetic curcumin analogs, among which tetrahydrocurcumin (THC), bis-o-hydroxycinnamoylmethane, bis-1.7-(2-hydroxyphenyl)- hepta-1.6-diene-3.5-dione (BDMC) analogs and two new curcumin derivatives C66 and B06 have been studied. All above-mentioned (mainly synthetic) curcuminoids have better solubility and bioavailability than curcumin.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Collection and Authentication

Fresh *Pilostigma reticulatum* and *Cocuma longa* leaves were collected from Biological Garden of the Federal University of Technology, Minna, Bosso Campus, Nigeria. It was identified and authenticated by a senior botanist of the Department of Plant Biology, Federal University of Technology, Minna. The selection of the plant parts of interest (leaves) was based on its usage by folklore people for curing diseases such as diabetes, fever, cancer, hepatitis, dysentery, hypertension, malaria, as well as, the organoleptic properties of the plants (Abdullahi *et al*., 2015).

3.2 Preparation of Plant Powder

After identification and authentication, the plants were washed immediately in tap water followed by distilled water (Abdullahi *et al*., 2015), and were then air-dried under shade at room temperature (about 2 weeks) until all the water content was lost completely and pulverized in to coarse powder using wooden mortar and pestle. The powder sample obtained was used to prepare the crude extracts.

3.3 Preparation of Crude Extracts

The total powdered sample weighed was two hundred grammes. The plant was extracted using cold maceration method of extraction.

Hundred grammes (100g) of the dried powdered form of the plant materials was weighed into a round bottom flask and 400ml of 100 % methanol was poured and shaken for proper mixture .The flask containing the mixture as alloed to stand for 48 hours after which it was filtered using clean muslin cloth into a sterile beaker and the resulting solution was concentrated using water bath at 40^0C and weighed. The extracts

was then stored in a clean universal containers and kept in a refrigerator at 4^0C to protect from light and moisture until use as described by Sutharson *et al*. (2007).

3.4 Phytochemical Screening Phytochemical Analysis of the Leaf Extracts

The Phytochemical Screening of the leaves extract; phenol, flavonoids, alkaloids, Tannins and saponins were determined according to the methods described by Singleton *et al*. (1999 & Sofowora 2008).

3.4.1 Determination of phenol

The 0.01g of the plant extracts of H.suaveolens as dissolved in 10ml of distilled water and 0.5 ml was oxidized by 25ml of 10%folin ciocalteu's reagent which was den neutralized by 2 ml of 7.5 sodium carbonate. The reaction mixture was incubated at 45oc for 40 mins. Absorbance was read at 765nm using double beam Shimaddzu UV spectrophotometer, UV-1800.Standard garlic acid was used to prepare the calibration curve. A dark green colour precipitate indicated the presence of phenolic compound (Singleton *et al.,* 1999)

3.4.2 Determination of Flavonoids

The plant extract (0.05 mL) was added to a test tube containing 1.5ml of absolute methanol 0.1ML of 10% aluminium chloride ,0.1ml of sodium acetate and 2.8ml of distilled water and incubated at ambient temperature for 30minutes.The absorbance was read at 415 with double beam Shimadzu UV-spectrophotometer, UV-1800, . Standard quercetin was used to prepare the calibration curve. A yellow precipitate indicated the presence of flavonoids (Singleton *et al*., 1999).

3.4.3 Determination of Tannins

0.2g of the plant extract was weighed into a 50 mL beaker and 50% methanol was added to it and covered with parafilm and heated in water bath at 80^0C for 1hour. The reaction mixture was shaken thoroughly to ensure uniformity. The extract as then

filtered into a 100mL of volumetric flask and 20ml of distilled water, 2.5mL of Folin-Denis'reagent and 10ml of sodium carbonate were added and mixed properly. The reaction mixture was then allowed to stand for 20 minutes at room temperature. The Absorbance was taken at 760nm using double beam Shimaddzu UV spectrophotometer, UV-1800.Standard tannic acid was used to prepare the calibration curve. A Blueish-green colour precipitate indicated the presence of Tannins compound (Singleton *et al.,* 1999).

3.4.4 Determination of Alkaloids

0.5gram of the plant extract as weighed and dissolved in 5ml of mixture of 96% ethanol: 20% H_2SO4 and then filtered. 1ml of the filterate was then added to a test tube containing containing 5ml of the 60%H_2SO4 and allowed to stand for 5minutes. Thereafter, 5ml of 0.5% formaldehydes was added and allowed to stand at room temperature for 3 hours. The absorbance was read at wavelength of 565nm.A red precipitate indicated the presence of alkaloids (Singleton *et al.,* 1999).

3.4.5 Determination of saponins

0.5gram of the plant extract as weighed and dissolved in 20mL of HCL and boiled in water bath at 80^0C for 4hours.The reaction mixture was cooled and filtered .50ml of petroleum ether was added and the ether layer was collected and evaporated to dryness. Thereafter, 5ml of acetone ethanol, 6mL of ferrous sulphate and 2ml of concentrated sulphuric acid were added and allowed to stand for 10 minutes .The absorbance was taken at 490nm.standard saponin was used to prepare the calibration curve. A stable froth indicated the presence of saponnins (Singleton *et al.,* 1999).

3.5 Entomological bioassay

3.5.1 Preparation of stock and working solutions of extracts

Stock solutions of the plant extracts were prepared according to World Health Organisation (WHO) protocols (WHO, 2005) with slight modification. Briefly, for stock solution of methanolic, ethyl acetate and n-hexane extracts of the plants, 1 g of the extracts were dissolved in 10 ml of methanol, ethyl acetate and n-hexane. Working solution was, thereafter, prepared by adding 1 ml of stock solution to 99 mls of distilled water. Test concentrations of 0.1, 0.2 and 0.3 mg/L and 0.1, 0.25, and 0.5 mg/L, respectively, of the n-hexane and methanolic extracts were prepared by respectively adding 0.1, 0.2, 0.5, 1, 2.5, and 5 ml of working solution to 99.875, 99.75, 99.95, 99, 97.5, and 95 ml of distilled water

3.5.2 Collection of mosquitoes larvae

Collection of mosquito larvae was carried out three times in a day, in stagnant water located in Bosso market (Federal Ministry of Health, 2005; 2014). The sites were visited between 6am and 9am, and 6pm.A yellow bowl or scoop was used to collect the water in a stagnant water and the water was taken to biology laboratory and allowed to settle so that the mosquito larvae will come up after which a pipette was used to remove all the mosquito larvae that were at the third and fourth instar stage. The larvae were immediately preserved in a rubber for rearing according to WHO (1968).There were kept at room temperature and fed with powered yeast

3.5.3 Preparation of stock and working solution

The stock and working solutions of the plant extracts were prepared following the department of biological sciences developed protocols for testing mosquito larvicides. 1 g of crude extract was 100 % concentrated. 1g of crude extract was dissolved in 10 mls of solvent of extraction (to get the stock solution), 10 % concentration of original

extract was made. 1 ml of stock solution was added to 99 mls of distilled water to get working solution; this resulted in 0.10 % concentration of original extract. For test concentrations of 0.1, 0.5, 1.0, 2.0 mg/L (i.e., 1, 5, 10, 20 ppm, respectively), graded volumes of working concentration were added to 99, 95, 90, 80mls of distilled water respectively.

3.5.4 Bio-assay of Extracts against Mosquito Larvae

The bio assay was carried out in biological laboratory II Federal University of Technology under laboratory condition. Bio-assay of larvae was carried out based on W.H.O (2005) protocol with little modifications. Batches of 25 late third instar and early fourth instar larvae of the *Culex quinquefasciatus* mosquitoes were transferred to 250 mls bowl containing 100mLs of distilled water with different concentration of plant extract of testing solutions ranges from 0.1, 0.5, 1.0, 2.0, and 0.25% depending on result of initial pilot run. The concentration was used to treat larvae under ambient laboratory conditions. For each treatment, four replicates were set up and a concurrent Control group was also set up with four replicates (plate III). After treatment, changes in treated larvae were observed at various time intervals: 0, 5, 10, 15, 20, 30, minutes, 1, 2, 3, 6, 12, 18, 24 hours. Death of moribund larvae were determined when pricked with a sharp object and larvae refused to move, and were removed from the treatment bowl using rubber pipette.

3.5.5 Bioassay of plant extracts against Fourth instar larvae of *Culex quinquefasciatus*

The mosquitoes were exposed to the extracts following standard World Health Organisation's Protocols for testing the efficacy of insecticides (WHO, 2005), with slight modifications. For the methanolic extracts, batches of 4th instar larvae of Cx. quinquefasciatus were separately exposed to 0.10, 0.30, and 0.50 mg/L of the extracts in

250 mL capacity bowls. There were two Controls namely, positive and negative, containing 1% methanol-distilled water, and only distilled water (i.e., neither extract nor solvent), respectively. Each test concentration and Controls had five replicates. The n-hexane extract bio-assay had the same set-up as the methanolic extract counterpart, except that the test concentrations were 0.1, 0.2 and 0.3 mg/L, and the positive control was 1%n-hexane-distilled water. The experiments were maintained in the Laboratory at ambient conditions of 28.00±1.00^{0}C, 70.20±2.63 % RH, and 12:12 hours (L: D). The larvae were monitored and mortality recorded after 24 hours of exposure

3.6 Data Analysis

LC_{50} and LC_{90} were determined using Probit analysis, larval mortality was expressed as mean± standard error of mean, difference in mortality among the test concentrations were determined using Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) was used to separate the mean.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Qualitative phytochemical constituents of *Pilostigma reticulatum* and *Cocuma longa.*

The result of the qualitative phytochemicals present in *Pilostigma reticulatum* and *Cocuma longa* is represented in Table 4.1. The results indicated the presence of bioactive metabolites including flavonoids, phenols, alkaloids, tannins in both plant crude methanol extracts. Cardiac glycoside and anthraquinones were both absent in *Cocuma longa.*

Table 4.1 Qualitative Phytochemical Constituents of *P. reticulatum*and *C. longa* Crude Extracts.

Phytochemicals	*Pilostigmareticulatum*	*Cocuma longa*
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Alkaloids	+	+
Steroids	+	+
Terpenoids	+	+
Cardiac glycosides	+	-
Anthraquinones	+	-

Keys: + = Present, – = Absent

4.1.2 Quantitative phytochemical composition of *Pilostigma reticulatum* and *Cocuma longa.*

The result of the quantitative phytochemical constituents of *Pilostigma reticulatum* and *C. longa* is presented in Table 4.2. In both plant saponins contents were significantly higher ($p < 0.05$) than other phytochemicals observed. While *P. reticulatum* has higher phenolic contents (241.77 ± 1.31 mg/100g) than *C. longa* (189.31 ± 0.14), flavonoids (231.32 ± 0.32) and alkaloids was higher in *C. longa* than in the *P. reticulatum.*

Table 4.2: Quantitative Phytochemical Constituents of *Pilostigma reticulatum* and *C. longa*

Phytochemicals	*Pilostigma reticulatum*(mg/100g)	*Cocuma longa* (mg/100g)
Phenols	241.77 ± 1.31^{d}	189.31 ± 0.14^{c}
Flavonoids	201.32 ± 0.08^{c}	231.32 ± 0.32^{d}
Tannins	87.11 ± 2.14^{b}	98.14 ± 0.08^{b}
Saponins	321.10 ± 1.34^{e}	401.49 ± 0.91^{e}
Alkaloids	49.28 ± 0.56^{a}	84.23 ± 0.45^{a}

4.1.3 Larvicidal activities of methanol extract of *Pilostigma reticulatum* against 4th instar larval of *Culex quinquefasciatus.*

The result of the larvicidal activities of crude methanol extract of *P. reticulatum* against 4thinster larvae of *Culex quinquefasciatus* is presented in Table 4.3. On the general note, the result showed that, in the first 15minutes, the extract at all concentration recorded no larvae mortality. Thereafter, larvae mortality increases with increase in time of exposure and significantly ($p<0.05$) increase with increase in extract concentration. The highest mortality was recorded at 1.0mg/L extract concentration compared to both positive and negative control where no mortality was recorded.

Table 4.3 Larvicidal activities of methanol extract of *Pilostigma reticulatum* against 4th instar larval of *Culex quinquefasciatus*

Progressive larvicidal activities ($\overline{X}$individual ± *Sf*)

Conc. mg/L	0min	5mins	10mins	15mins	30mins	1hour	3hours	6hours	12hours	24hours
0.2	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	2.12±0.11[b]	2.00±0.11[b]	2.00±0.11[b]	2.00±0.11[b]	3.15±0.14[b]	3.15±0.14[b] (12.6)
0.4	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	2.41±1.10[b]	3.10±0.78[c]	3.10±0.78[b]	4.51±0.12[c]	6.21±0.03[c]	6.21±0.03[c] (24.84)
0.6	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	2.85±0.03[b]	3.14±0.14[c]	5.43±0.10[c]	6.14±1.25[d]	8.25±0.07[d]	10.14±0.07[d] (40.56)
0.8	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	3.41±0.13[c]	10.40±0.14[d]	11.71±0.3[d]	13.02±0.11[e]	15.13±0.17[e]	17.21±1.30[e] (65.84)
1.0	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	7.32±0.39[d]	10.98±1.12[d]	13.41±0.32[e]	14.10± 0.04[e]	16.34±0.07[e]	18.13±0.89[e] (72.52)
Positive control	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Negative control	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]

4.1.4 Larvicidal activities of the crude methanol extracts of *Cucuma longa* against 4th instar larvae of *Culex quinquefasciatus*

The result of the larvicidal activities of the crude methanol extract of *C. longa* against 4th instar larvae of *Culex quinquefasciatus* is represented in Table 4.4. In the first 30minutes of the experiment, no larvae mortality was recorded for all the tested concentration. At 1 hour exposure period, only the groups subjected to 1.0mg/L extract concentration recorded larvae mortality. As extract concentration increases from 3hours to 24hours exposure period, larvae mortality increased significantly ($p<0.05$) with increase in extract concentration. The highest larvae mortality was recorded in treatment exposed to 1.0mg/L extract concentration.

Table 4.4 Larvicidal activities of the crude methanolic extract of *C. longa* against 4th instar larvae of *Culex quinquefasciatus*

Progressive Larvicidal activity ($\bar{X}\pm$ ***S.E.*** Individuals)

Conc. (mg/L)	0min	5mins	10mins	15mins	30mins	1hour	3hours	6hours	12hours	24hours
0.2	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.18 ± 1.12^{b}	2.18 ± 1.12^{b}	2.18 ± 1.12^{b} (8.72)
0.4	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.05 ± 0.05^{b}	3.21 ± 0.14^{b}	4.32 ± 0.04^{c}	8.20 ± 0.11^{c} (32.84)
0.6	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	5.12 ± 0.12^{c}	9.00 ± 1.15^{c}	13.23 ± 1.87^{d}	15.11 ± 0.87^{d} (60.44)
0.8	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	8.71 ± 0.05^{d}	10.21 ± 0.12^{c}	14.15 ± 0.69^{d}	17.12 ± 0.15^{e} (68.48)
1.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	3.41 ± 0.05^{b}	11.15 ± 0.90^{e}	14.40 ± 0.08^{d}	17.83 ± 1.11^{e}	19.14 ± 0.05^{f} (76.56)
Positive control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Negative control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

4.1.5 Larvicidal activities of N-hexane, Ehtylacetate and Methanol fractions of *Pilostigma reticulatum* against 4th instar Larvae of *Culex quinquefasciatus* after 24 hours exposure.

The result of the larvicidal activities of solvent fractions of *Pilostigma reticulatum* against 4th instar larval of *Culex quinquefasciatus* after 24 hours exposure period is contained in Table 4.5. For all the fractionated extracts, it was found that larvae mortality increased significantly ($p < 0.05$) with increase in extract concentration. Among the solvent fraction tested, N-hexane was found to show higher larvae mortality recording 89.24% larvae mortality. Mortality recorded was low for methanol fraction compared with ethylacetate fraction that recorded more than 80% mortality (88.48%).

Table 4.5 Larvicidal activities of N-hexane, Ehtylacetate and Methanol fractions of *Pilostigma reticulatum* against 4th instar Larvae of *Culex quinquefasciatus* after 24 hours exposure.

Extract concentration (mg/L)	N-hexane (%)	Ehtylacetate (%)	Methanol (%)
1.0	2.12 ± 0.04^{b} (8.48)	3.21 ± 0.86^{b} (12.84)	2.00 ± 0.34^{b} (8.00)
2.0	4.41 ± 0.32^{c} (17.64)	4.00 ± 0.35^{b} (16.00)	3.64 ± 0.11^{b} (14.56)
3.0	17.28 ± 1.14^{d} (69.12)	6.53 ± 0.91^{c} (26.12)	4.53 ± 0.31^{c} (18.12)
4.0	22.31 ± 0.56^{e} (89.24)	13.12 ± 0.84^{d} (52.48)	9.30 ± 0.05^{d} (37.24)
5.0	25.14 ± 0.00^{f} (100.00)	22.12 ± 0.31^{e} (88.48)	11.16 ± 0.32^{e} (44.64)
Positive control	00.00 ± 00.00^{a} (00.00)	00.00 ± 00.00^{a} (00.00)	00.00 ± 00.00^{a} (00.00)
Negative control	00.00 ± 00.00^{a} (00.00)	00.00 ± 00.00^{a} (00.00)	00.00 ± 00.00^{a} (00.00)

4.1.6 Larvicidal activities of N-hexane, Ehtylacetate and Methanol fractions of *Cocuma longa* against 4th instar Larvae of *Culex quinquefasciatus* after 24 hours exposure.

The result of the larvicidal activities of solvent fractions of *C. longa* against 4th instar larval of *Culex quinquefasciatus* after 24 hours exposure period is contained in Table 4.6. On the general note, the result indicated a significant larvae mortality ($p<0.05$) with increase in the extract concentration when compared with the control. Both N-hexane and ethylacetate fractions recorded 100% mortality. On the other hand, mortality was significantly lower for the group exposed to methanol fraction of the plant crude extract. No larvae mortality was recorded for the positive and negative control group.

Table 4.6 Larvicidal activities of N-hexane, Ehtylacetate and Methanol fractions of *Cocuma longa* against 4th instar Larvae of *Culex quinquefasciatus* after 24 hours exposure.

Mortality rate (X ± S.E)

Extract concentration (mg/L)	N-hexane (%)	Ehtylacetate (%)	Methanol (%)
1.0	7.14±0.46 (28.56)	6.10±0.12 (24.40)	2.13±1.04 (8.52)
2.0	9.23±0.11 (36.92)	11.21±1.11 (44.84)	4.14±0.01 (16.56)
3.0	13.14±0.01 (52.56)	12.45±1.21 (49.80)	6.23±0.07 (24.92)
4.0	23.01±2.00 (92.04)	20.00±0.49 (80.00)	8.04±0.98 (32.16)
5.0	25.00±0.10 (100.00)	25.00±0.00 (100.00)	10.51±1.01 (42.04)
Positive control	00.00±00.00[a] (00.00)	00.00±00.00[a] (00.00)	00.00±00.00[a] (00.00)
Negative control	00.00±00.00[a] (00.00)	00.00±00.00[a] (00.00)	00.00±00.00[a] (00.00)

4.1.7 Lethal concentration and coefficient of determination of the larvicidal activities of crude and fractions of *P. reticulatum* and *C. longa* against *Culex quinquefasciatus* after 24 hours Period of exposure.

The media (LC_{50}) and upper lethal (LC_{90}) concentration, coefficient of determination and regression equation of the larvicidal effects of the crude and solvent fractions of *P. reticulatum*and *C. longa* after 24 hours exposure period is presented in Table 4.7. In both extracts, the best LC_{50} and LC_{90} were recorded for the N-hexane fractions. The crude methanol extract of *P. reticulatum* recorded lower and better LC_{50} (0.67mg/L) and LC_{90} (1.16mg/L), respectively. Similarly, the crude methanol extract of *C. longa* showed better larvicidal activity (LC_{50}; 0.61mg/L) than the methanol fraction (LC_{50}; 1.21mg/L). The values of R^2(coefficient of determination) recorded for all the extracts and fractions was above 0.93, which implies that more than 90% of the extract was responsible for the mortality recorded.

Table 4.7 Lethal concentration and coefficient of determination of the larvicidal activities of crude and fractions of *P. reticulatum*and *C. longa* against *Culex quinquefasciatus* after 24 hours exposure.

Extracts	LC_{50}(mg/L)	LC_{90} (mg/L)	R	Regression Equation
*P. reticulatum*crude	0.67	1.16	0.9603	Y=81.92x−5.28
Fraction N-hexane	0.54	0.86	0.9340	Y=127.4x−19.576
Ethylacetate	0.72	1.25	0.8794	Y=93.88x−17.144
Methanol	1.13	1.97	0.9401	Y=47.96x−4.272
C. longa crude	0.61	1.07	0.9310	Y=85.66x−1.988
Fraction N-hexane	0.47	0.88	0.9373	Y=99x+2.616
Ethylacetate	0.49	0.92	0.9644	Y=93.18x+3.9
Methanol	1.21	2.18	0.9980	Y=41.32x+0.048

4.2 Discussion

One of the novel strategies for controlling mosquito menace is the aggressive search for mosquito-larvicidal agents from natural product, especially plants. This is the main reason for assessing the larvicidal potency of *P.reticulatum* and *C. Longa*.

In the current study the photochemical screening revealed the presence of phytochemical's such as flavonoid, saponins, alkaloids, tannins and phenon in both *P. reticulatum* and C. longa. These compounds are referred to as plant secondary metabolites. According to Lumpkin (2005) and kumar *et al*., (2017), these phytochemicals are usually produced by plants during secondary metabolic activities as well as, in response and/or counter effect against plant bioactive metabolites from plants have been well established as pathogens templates for several synthetic drugs as well as precursors for the production of semi synthetic drugs (Wubshet and Endathachew, 2014). The study also recorded varying variations in the concentrations of the phytochemical observed with respect to the type of plant and solvent of extraction. The variation may be due to the differences in the polarity of the solvents. Other factors may be environmental factors such as geographical location which has an influence on soil type, light intensity and temperature (Audu *et al*., 2012).

In the current study, it was observed that both plant crude and solvent fractions showed dose dependent larvicidal activities. These observations are common to plant extracts with antiplasmodial efficiency. This is in line with the previous report of kim *et al.,* (2008) who recorded dose dependent larvicidal activities for Nuclear latifolia against different mosquito species. Increase in extract concentration implies increase in the bio-active metabolic of the medium. This will therefore increase the toxicity against the mosquito larvae.

The increase in larval mortality with increase in exposure period observed in the current study is an indication that the bioactive compounds have more time to cause the significant mortality. This corroborate the previous report of Olayemi *et al*., (2014) who mentioned similar observation with *Jatropha curcas* larvicidal activities.

Both *P. reticulatum* and *C. longa* crude methanol extract show higher larvae mortality against the tested mosquito larvae, (*Cx. quinquefasciatus*) compared to the methanol fraction. The larvicidal potency of crude plant extracts is mostly attributed to the diversity of the plants inherent bioactive compounds. These bioactive compounds have been found by other scholars to be responsible for mosquito larvicidal activity (Shallan *et al*., 2015). Most studies have reported that the active larvicidal compounds in plants include saponins, terpenoids, flavonids and steroids (Olayemi *et al*., 2017). According to Chowdhury *et al*., (2008) and Bayavan *et al.* (2008), steroid and saponins are potential larvicidal compounds and are responsible for mosquito larval toxicity (Pelah *et al*., 2015).

According to the report of Raghavendra *et al*. (2001), these plant bioactive metabolite caused larval toxicity by interacting with the cuticular membrane of the larva, thereby disarranging the membrane, which is the main reason for larval death. Furthermore, these compounds may jointly on independently contribute to producing toxic activity against the tested mosquito species.

The observed difference in the larvicidal activity of the plant extracts could be attributed to different concentration of phytochemicals in the extracts. The activities demonstrated by the two plant extracts and the solvent tractions could also be attributed to the uneven distribution of chemical constituents within these extracts. By implication, the phytochemicals could either exhibited synergistic or additive effect on the tested

mosquito larvae. In addition, Elsheikha and khan (2011), most insecticide target the nervous system, endocrine and metabolic processes. The metabolic processes are inhibited by causing membrane disruption and mechanical suffocation other mechanism of action of other bioactive compound include promoting over stimulation of the nervous system which leads to paralysis and death. This indicates that the observed larvicidal activities may be due to the ability of the metabolism to inhibit cholinesterase activities and/or by acting sodium channel modulators (Brown, 2006).

In the current study, among the solvent fractions tested, n-hexane fraction showed higher larvae mortality than other fractions tested. It is possible that the active larvicidal metabolites may be more concentrated in the n-hexane fraction than other extract tested. Olayemi *et al*., (2017) reported variation in larval mortality with respect to the different solvent of extraction tested. Larvicidal activities of the extracts have been also attributed to the polarity of solvent of extraction (Adefolalu *et al*., 2015). This is however, not in conformity with the previous report of Rahuman *et al*. (2008) who reported that petroleum either possess higher larvicidal potency than other solvent tested.

Furthermore, the current study recorded that the n-hexane extract of *C. longa* was more potent against the tested mosquito with the lowest LC_{50} compared to the LC_{50} of the P. reticulation. This is an indication that the metabolites are more active in the C. longa than in the *p. reticulatum*. The LC_{50} recorded to *C. longa* and *p. reticulatum* were far better than those reported for other plants. Olayemi *et al*. (2014) recorded higher LC_{50} (2.65 mg/L) for the extract of *Jatropha curcas* against *Culex, pipiens* mosquito. Govindarajan, (2011), reported LC_{50} values ranging between 3.8 and 4.8g/L for the crude extract of *Cidaacuta* against *Cx. quinquefasciatus.*

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In conclusion, the study revealed that the crude extracts of *P. reticulatum* and *C. longa* contain bioactive insecticidal compounds including steroids, flavonoids, terpenoids, flavonoids, saponins and tannins. The two plants namely, *P. reticulatum* and *C. longa* possess larvicidal activity against *Cx. quinquefasciatus*. The larvicidal activities of the two plant extracts increases with increase in extract concentration and exposure period. Among the solvent fractions tested, n-hexane fraction was more potent than the ethylacetate fraction. Furthermore, the crude and fraction of *C. longa* was more potent against mosquito larvae than the crude and fractions of *P. reticulatum*.

5.2 Recommendations

i. There is need to subject the fraction with best larvicidal activities to further fractionation in other to purify and identify the active ingredient in the plants.

ii. Further investigation are needed to ascertain whether there are non-target effects of the extracts and fraction on other water inhabiting insects, especially the larvae predators.

iii. There is need to establish the mechanism of action of the extracts and especially the fraction that showed the best larvicidal potency.

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APPENDICES

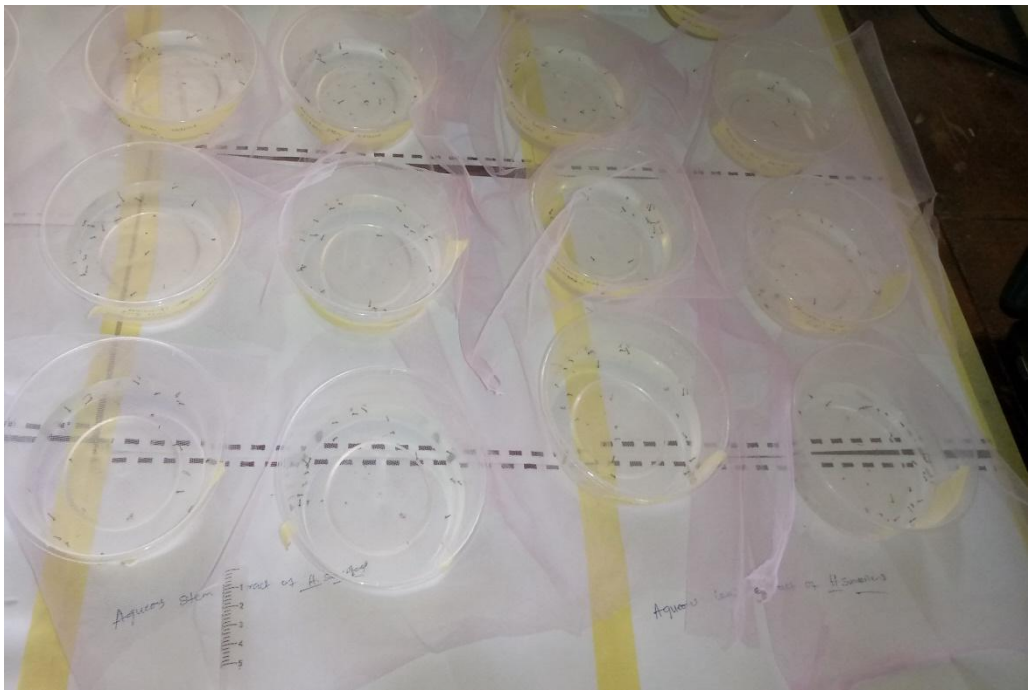

Plate I: Bioassay Setup