

**EFFECT OF SOME ETHNO-MEDICINAL PLANTS EXTRACTS ON *CULEX*
QUINQUEFASCIATUS (SAY)**

BY

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**DEPARTMENT OF ZOOLOGY,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA.**

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ZARIA, NIGERIA

JANUARY, 2017

DECLARATION

I declare that the work in this thesis entitled “**EFFECTS OF SOME ETHNO-MEDICINAL PLANTS EXTRACTS ON *CULEX QUINQUEFASCIATUS* (SAY)**” has been carried out by me in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The information derived from the literature has been duly acknowledged in the text and in a list of references provided. No part of this thesis was previously presented for another Degree or Diploma in this or any Institution.

SAIDU Yahaya Ubandoma

Signature

Date

CERTIFICATION

This thesis entitled EFFECTS OF SOME ETHNO-MEDICINAL PLANTS EXTRACTS ON *CULEX QUINQUEFASCIATUS* (SAY) meets the regulations governing the award of the Degree of Doctoral Degree in Zoology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to the development of science worldwide.

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ABSTRACT

Studies on the ethanolic and aqueous leaf extracts of some ethno-medicinal plants (*Annona senegalensis*Pers,*Hibiscus lunariifolius*Willd, *Hibiscusasper*Hook.f., *Vernonia amygdalina*, *Hibiscuscannabinus*Linn, *H. Sabdariffa*Linn, *Urenalobata*Linn, *H. esculentus* Linn.) for the control of *Culex quinquefasciatus* was carried out from December, 2013 to July, 2016, in Zaria metropolis, Kaduna State, Nigeria. Specimens of the selected plants were obtained from Zango, Hanwa, Samaru, Bomo, Danmagaji and Dakache areas and identified by Herbarium Keepers in the Herbarium Unit of the Department of Botany, Ahmadu Bello University Zaria and voucher numbers given. Fractions of the leaf extracts were separated using Thin Layer Chromatography (TLC) and Column chromatography; and the active compounds were characterized by Nuclear Magnetic Resonance (NMR) spectroscopy. Secondary metabolites recovered from the leaf extracts include sterols, flavonoids, saponins and tannins. Anthracenes and combined anthracenes were present only in the leaf extracts of *Annona senegalensis* and *Vernonia amygdalina* except anthraquinones. Gravid females of *Culex quinquefasciatus* were collected from 6:00 am to 8:00 am in the morning from classrooms of the Faculty of Science and Education, ABU, Zaria. They were introduced into rearing cages and provided with oviposition bowls containing water for egg laying. The eggs, laid in rafts hatched into 1st instar Larvae which were fed on Baker's Yeast once a day initially, and twice during the later stages of the development. The larvicidal bioassay followed the World Health Organization (2005) standard protocols in "Guidelines for laboratory and field testing of mosquito larvicides". A narrower range of 4 concentrations (10.00mg/ml, 5.00mg/ml, 2.50mg/ml and 1.25mg/ml) was prepared and batches of 25 4th larval instar

were introduced in four replicates. Probit analysis and one way ANOVA were used to determine LC₅₀, larval mortality and inhibition of adult emergence. Student t-test was employed to determine the difference between ethanolic and aqueous leaf extracts of *A. senegalensis*. Similarly, t-test was used to analyze differences in oviposition parameters between *Culex quinquefasciatus* adults reared in ethyl acetate leaf fraction (LC₅₀, 3.7 mg/ml) of *A. senegalensis* and those reared in water (control). The more effective extracts comprised *A. senegalensis* (LC₅₀: 0.457), *Vernonia amygdalina* (LC₅₀: 0.536), *Hibiscus esculentus* (LC₅₀: 0.571), and *Urena lobata* (LC₅₀: 0.625). However, *A. senegalensis* leaf extract was the most effective. The least effect was observed in *Hibiscus asper* leaf extract with the LC₅₀ of 4.032. Moderate effectiveness was observed in *Hibiscus cannabinus*, *Hibiscus sabdariffa* and *Hibiscus lunariifolius* extracts with LC₅₀: 1.237, LC₅₀: 2.193 and LC₅₀: 2.568, respectively. The larvicidal activity of the ethanolic leaf extract was higher than that of the aqueous extract at all concentrations, especially, at the highest concentration, 10 mg/ml (24.25/25 or 99% and 7.00 or 28%, respectively). Ethanol dissolves bipolar compounds that have insecticidal properties. The effects of the leaf extract of *A. senegalensis* on larval histopathology resulted in apolysis of the head cuticle, separation of the basement membrane from the midgut epithelium and the degeneration of the microvilli. There was reduction in developmental stages of *Culex quinquefasciatus* as there was 101 viable eggs produced in the control compared to 70 recorded in the one laid by mosquitoes that survived treatment. The percentage of female to male in the control was 49% and 51%, respectively; while ethyl acetate fraction was 32% for female and 68% for male. The differences in the means were significant in all cases except in the sex ratios. Bioassay with ergosterol, the isolated compound, recorded

80.00% larval mortality at dose 1000 µg/ml. In consideration of its high activity, it can, therefore, be suggested that ergosterol, might be responsible for the mortality observed in 4th instar larvae, histological deformities, inhibition of emergence of survived larvae, reduction in reproductive ability of survived adult, low egg count and hatching success, and reduction in sizes of the second filial generation (F2) of *Culex quinquefasciatus*. Ergosterol is a component of cell membrane of eukaryotes and in peroxidestate; it has been shown to cause disruption of cellular constituents. Ergosterol has been reported to have cytotoxicity and antimicrobial effects. The results of this study suggest that leaf extracts and leaf fractions of *A. senegalensis*, *Vernonia amygdalina*, *Urena lobata* and *Hibiscus esculentus* contain sterols like ergosterol which have insecticidal properties. The formulation of ergosterol as insecticide may contribute immensely to the control of mosquito vector diseases.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

The World Health Organization(1996) has declared mosquitoes as “public enemy number one” because mosquitoes transmit more diseases than any other group of arthropods and affect millions of people throughout the world. Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people every year worldwide (Govindarajan and Rajeswary, 2014). They act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, and West Nile virus infection, in tropical and subtropical countries and many other parts of the world (Anirud *et al.*, 2013).

The mosquito has been described as the most dangerous animal in the world and the mosquito-borne disease with the greatest detrimental impact is undoubtedly malaria. There are about 3,500 mosquito species and those that transmit malaria all belong to a sub-set called the *Anopheles*. Approximately 40 *Anopheles* species are able to transmit malaria well enough to cause significant human illness and death (Russellet *al.*, 2009).

Culex quinquefasciatus(Say, 1823), commonly known as the southern house mosquito, is a medium-sized brown mosquito that exists throughout the tropics and the lower latitudes of temperate regions.This nighttime-active, opportunistic blood feeder is a vector of many pathogens, several of which affect humans. *Culex quinquefasciatus* is the primary vector of St. Louis encephalitis virus (SLEv) and also transmits the West Nile virus (WNV).Also,*Culex quinquefasciatus*transmit 90% of filarial infections caused by*Wucheraria bancrofti*. Adults are generally active only during the warmer months;

they usually attack humans towards the middle of the night in both indoors and outdoors, but are often more attracted to birds (Ajaiyeoba *et al.*, 2006; Anirud *et al.*, 2013).

To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation is the application of synthetic insecticides such as organochloride and organophosphate compounds (Soderlund, 1995). But this has not been very successful due to human, technical, operational, ecological and economic factors. In recent years, use of many of the former synthetic insecticides in mosquito control programme has been limited. This is due to lack of novel mosquitocides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health and other non-target populations (Soderlund, 1995). Other limiting factors include non-biodegradable nature, higher rate of biological magnification through ecosystem, and increasing insecticide resistance on a global scale (Brown, 1986; Russel *et al.*, 2009).

The biological control of immature stages now appears to be the most powerful means of reducing target populations of *Culicidae* and other dipteran pests. This has prompted researchers to look for alternative approaches ranging from provision/promoting the adoption of effective and transparent mosquito management strategies that focus on public education, monitoring and surveillance, source reduction and environment friendly least-toxic larval control. These factors have resulted in an urge to look for environment friendly, cost-effective, biodegradable and target specific insecticides against mosquito species. Considering these, the application of eco-friendly alternatives such as biological

control of vectors has become the central focus of the control programme in place of the chemical insecticides (Alawaet *al.*, 2003).

One of the most effective alternative approaches under the biological control programme is to investigate the floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple and sustainable method of mosquito control. Insecticides of plant origin have been extensively used on agricultural pests, and to a very limited extent, against insect vectors of public health importance, which deserve careful and thorough screening. The use of plant extracts for insect control has several appealing features, as these are generally more biodegradable, less hazardous than chemical insecticides, and a rich storehouse of chemicals of diverse biological activity (Alveset *al.*, 2004). In addition, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise botanical blends of chemical compounds which act concertedly on both behavioural and physiological processes of the organism. Thus, there is very little chance of pests developing resistance to such compounds. Identifying bio-insecticides that are efficient, as well as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management (Alveset *al.*, 2004).

Plants produce numerous chemicals, many of which have medicinal and pesticidal properties. More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programmes. Members of some plant families Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae

have various types of larvicidal, adulticidal or repellent activities against different species of mosquitoes (Shaalane *et al.*, 2005).

Botanicals are basically secondary metabolites that serve as a means of defence mechanism of the plants to withstand the continuous selection pressure from herbivores 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 (Shaalane *et al.*, 2005). 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. A wide selection of plants from herbs, shrubs and large trees was used for extraction of mosquito toxins. Phytochemicals were extracted either from the whole body of little herbs or from various parts like fruits, leaves, stems, barks, and roots of larger plants or trees. In all cases the most toxic substances were concentrated upon, found and extracted for mosquito control (Alawaet *et al.*, 2003).

Insecticides of plant origin have been extensively used on agricultural pests, and to a very limited extent, against insect vectors of public health importance, which deserve careful and thorough screening. The use of plant extracts for insect control has several appealing features, as these are generally more biodegradable, less hazardous than chemical insecticides, and a rich storehouse of chemicals of diverse biological activity (Nath, Bhuyan and Goswami, 2006).

Currently, several products of botanical origin, especially, phytoecdysteroids, have received significant renewed attention as potentially bioactive agents used in insect vector management. Phytoecdysteroids affect behavioural and physiological processes.

Hence, the chances of pests developing resistance to such substances are less likely. Moreover, they are less likely to bioaccumulate as they are biodegradable. Phytoecdysteroids represent analogues of insect steroid hormones (ecdysteroids) that control insect growth, development, and reproduction. It has been suggested that they form part of the plant's defences against phytophagous insects and soil nematodes (Sayah and Lafont, 2010).

The exogenous application of phytoecdysteroids to a number of species resulted in marked growth and developmental disruption, e.g. in *Spodoptera frugiperda*, *Bombyx mori*, *Lobesia botrana*, *Inachis io* and *Aglais urticae*, and *Bradysia impatiens*. This disruption involves a number of effects including inhibition of growth, induction of supernumerary larval instars, death without moulting, and death during or after induced moulting (Rharrabe *et al.*, 2010).

Insect growth and development from one stage to another is regulated by two main hormones, the steroidal insect moulting hormone, 20-hydroxyecdysone (20E) and the sesquiterpenoid Juvenile Hormone (JH). As an insect embryo grows, it undergoes embryonic moults, where each moult is regulated by 20E. The moulting process continues through the larval and pupal stages culminating into the adult stage. While moulting to accommodate growth is regulated by 20E, the development from an egg to larva to pupa to adult is regulated by the timing and titers of JH, according to Riddiford (1996). In the adult stage, both these hormones, being pleiotropic, change their roles to regulating reproductive processes (Wyatt and Davey, 1996).

One of the first effects of phytoecdysones upon ingestion by susceptible larvae is feeding inhibition. During this time, synthesis of a new cuticle begins and apolysis of the new cuticle from the old one takes place. Subsequently, the intoxicated larvae become moribund, slip their head capsule, and, in extreme cases, the hind gut may be extruded (Dhadialla *et al.*, 2005). The new cuticle is not tanned or sclerotized. One resulting consequence is that the mouth parts under the slipped head capsule remain soft and mushy, thus, preventing any crop damage even if the head capsule comes off from mechanical or physical force. The larvae ultimately died as a result of their inability to complete a moult, starvation, and desiccation (Dhadialla *et al.*, 2005).

The reason for the lethal precocious moult effects is that the larva produces a new cuticle which is malformed. Unlike during normal cuticle synthesis, the lamellate endocuticle deposition in phytoecdysone-intoxicated larvae is disrupted and incomplete. The epidermal cells in intoxicated larvae have fewer microvilli; show hypertrophied Golgi complex and an increased number of vesicles compared to normal epidermal cells active in cuticle synthesis (Dhadialla *et al.*, 2005).

1.2 Statement of the Research Problem

To the best of my knowledge, the effect of the extracts and fractions of the selected plants on survival, morphology and oviposition in *Culex quinquefasciatus* are not reported. Similarly, the active chemical compound(s) that might be responsible for the activity of the selected plants has not been characterised.

1.3 Justification

Mosquito resistance to specific insecticides has reduced the effectiveness of chemical control and this has prompted the need for alternative means of vector population control, hence, the reduction in disease transmission. Many undiscovered plants species have good potential pesticides efficiency that can serve as suitable tool in controlling mosquito. Indeed, discovery of active larvicidal phytochemicals would provide a better effective way to alleviate the problem of insecticide resistance that has become a problem in mosquito control (Fatallah, 2010; Ajaiyeoba *et al.*, 2006).

1.4 Aim

To evaluate the efficacy of some selected ethno-medicinal plants found in Zaria local government area, Kaduna State, Nigeria, on *Culex quinquefasciatus*.

1.5 Objectives

The objectives of this work were to:

- i. determine the phytochemical constituents in the crude leaf extracts of the selected plants
- ii. determine the larvicidal effects of the leaf extracts of the selected plants on *Culex quinquefasciatus*
- iii. determine the effects of ethanolic leaf extracts of the selected plants on the morphology and histology of *Culex quinquefasciatus* larvae
- iv. determine the effects of the plant fractions on the inhibition of adult emergence (IE%) and oviposition in *Culex quinquefasciatus*

- v. characterize the active compound(s) that may be responsible for the activity of the selected plants

1.6 Hypotheses

- i. There are no phytochemical constituents in the leaf extracts of the selected plants.
- ii. The leaf extracts of the selected plants have insignificant larvicidal activity on *Culex quinquefasciatus*.
- iii. The leaf extracts of the selected plants do not cause any morphological and histological defects on the larvae of *Culex quinquefasciatus*.
- iv. The leaf fractions of the selected plants have insignificant inhibitory effects on the emergence of adults or oviposition in emerging adults of *Culex quinquefasciatus*.
- v. The leaf fractions of the selected plants do not contain any active compounds.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mosquitoes

Mosquito species differ in the type of aquatic habitats they prefer for oviposition based on location, the physicochemical condition of the water body, and the presence of potential predators (Shililu *et al.*, 2003; Piyaratne *et al.*, 2005). Physico-chemical factors that influence oviposition, survival, and the spatio-temporal distribution of important disease vector species include salts, dissolved organic and inorganic matter, degree of eutrophication, turbidity, presence of suspended mud, presence or absence of plants, temperature, light and shade, and hydrogen ion concentration (Mogi, 1978; Amerasinghe *et al.*, 1995; Gimnig *et al.*, 2001). Understanding how these factors affect the distribution of a particular vector species and how they influence larval abundance is an essential component of larval biology and of great importance in the design and implementation of integrated vector management plans (Gimnig *et al.*, 2001).

2.2 Biology of Mosquito

Mosquitoes have a holometabolous type of development; that is, having four distinct stages in their lifecycle: egg, larva, pupa, and adult (Rueda, 2008). Larvae and pupae of mosquitoes require an environment with standing or flowing water for proper development. The female adult lays either single eggs (e.g., *Aedes*, *Anopheles*) or in clusters (e.g., *Culex*, *Culiseta*), up to several hundred at a time, on the surface of the water, on the upper surface of floating vegetation, along the margins of quiet water pools, on the walls of artificial containers or in moist habitats subject to flooding. The larvae (called

wrigglers) undergo shedding (or molting) of the skin (exuviae) four times before becoming pupa (Washington State Department of Health, 2008).

Larvae of most species usually filter out and feed on organic matter and other microorganisms in the water for about 1–3 weeks or longer depending on the water temperature. Larvae of mosquito predators (e.g. *Toxorhynchites*, *Lutzia*) feed on larvae of other mosquitoes. In some predatory species, the first instar is a filter feeder, and the predaceous feeding structures are not developed until the second instar. The pupae (called tumblers), or resting stage, appear after the fourth larval moult. Unlike larvae, pupae do not feed and live for 1–3 days before becoming adults (Washington State Department of Health, 2008).

Only adult female mosquitoes bite humans and animals. Male mosquitoes feed primarily on flower nectars, while the females require the blood meal to produce viable eggs. Some species (anthropophilous) prefer to feed on man, while others (zoophilous) feed in nature on animals (including mammals and birds) other than humans. Females of *Toxorhynchites* and other mosquitoes do not feed on blood. There are some species that readily feed on fish exposed to air, reptiles, amphibians, and insect larvae (Harris *et al.*, 1969; Harwood and James 1979). Some autogenous females can also produce viable eggs, even without blood meal. Females typically feed every 3–5 days, and in a single feeding a female usually engorges more than its own weight of blood.

Some species of mosquitoes (e.g., *Anopheles*) prefer to feed at dusk, twilight or nighttime, while others bite mostly during the daytime (e.g., *Aedes*). Other species exhibit seasonal switching of hosts that provides a mechanism for transmitting diseases from

animals to humans (called zoonotic disease transmission). Diapause (i.e., hibernation, aestivation, overwintering) occurs in various life stages, e.g. as eggs in *Psorophora* and most *Aedes*; as larvae in *Coquillettidia*, some *Culiseta*, *Mansonia*, *Orthopodomyia*, *Toxorhynchites*, *Wyeomyia*, some *Aedes*; as adults, fertilized females, in *Uranotaenia*, most *Culex*, some *Anopheles* and other *Culiseta*; and as either eggs or larvae in *Culiseta morsitans* (Theobald) (Stojanovitch and Scott, 1997).

The stimuli or factors that attract mosquitoes to a human or animal host are complex and are not fully understood. Mosquitoes, like other biting arthropods, use visual, thermal, and olfactory stimuli to locate a host (Rueda, 2008). Olfactory cues may be the most important as a mosquito nears the host but visual stimuli seem important for in-flight orientation, particularly over wider ranges. For daytime biters, movement of the host may initiate orientation toward that person or animal. Out of more than 300 compounds that are released from the human body as by-products of metabolism, more than 100 volatile compounds can be detected from human breath. Carbon dioxide is released primarily from the breath and the skin, and can be detected by mosquitoes. Carbon dioxide and octenol are common attractants that are used in monitoring and surveillance of mosquitoes in their habitats (Rueda *et al.*, 2001).

The antennae of mosquitoes have chemoreceptors that are stimulated by lactic acid, but can be inhibited by repellents. Repellents (e.g., DEET or N, N-diethyl-3-methyl-benzamide) are effective personal protective measures against biting insects to reduce or prevent transmission of vector-borne diseases (Rueda *et al.*, 1998).

Mosquitoes serve as diverse disease vectors for vertebrate animals including humans. Therefore, there is need for various control methods to stop their potentially devastating effects. However, these efforts require additional research pertaining to the biology of these organisms. Larval stage mosquitoes are much more abundant than adult mosquitoes and are also quite distinct in both form and function (Linser *et al.*, 2009).

The midgut of *Culex quinquefasciatus* larvae is divided into four regions (i.e. cardia, gastric caeca, anterior and posterior midgut). In all of these regions, the epithelium is composed of a single layer of columnar digestive cells that consists of apical microvilli, cytoplasm with numerous mitochondria and nucleus with polytene chromosomes. Regenerative cells are distributed alone or in groups scattered at the base of the digestive cells. The midgut epithelium is lined by a well-developed Peritrophic Matrix (PM) that separates it from the ingested food (Clements, 1996).

In mosquitoes, the fat body is distributed as a layer of cells close to the body wall, throughout the thorax and abdomen and as lobes which extend into the body cavity, or as sheaths around certain organs (Clements, 1996). The insect fat body is the major organ of intermediary metabolism. In addition to lipid synthesis, most haemolymph proteins are also synthesized in the fat body which also stores proteins, lipids and carbohydrates (Chapman, 1998). The total carbohydrate and lipid content of young adult mosquitoes provides a measure of the energy reserve accumulated during the larval stage and protein synthesis for the haemolymph in larvae and adults. However, in adult females, the fat body produces the yolk polypeptides (Clements, 1996). Several studies have suggested that, the fat body also plays a role in the immune response of the organism. Defensins are

proteins produced in the fat body, released into the haemolymph, possess 3 or 4 disulfide bounds and are active in the immune response against certain bacteria, yeast and viruses (Zanetti *et al.*, 1997; Hoffmann *et al.*, 1999).

The anterior midgut pH of mosquitoes varies in different species, but is generally maintained between 10.5 and 11 (Clements, 1996). A decrease in the midgut pH from an alkaline to a neutral value suggests an electrical imbalance, since the maintenance of alkaline pH is the main energy expense of the larvae.

Pyrethroids and DDT have been shown to affect sodium channels in mosquitoes (Bregues *et al.*, 2003). Deltamethrin acts on the nervous system of these arthropods, which leads to rapid paralysis and death (Haug and Hoffman, 1990). Organophosphates inhibit acetylcholinesterase-mediated acetylcholine hydrolysis, resulting in an acute toxic effect due to hyperstimulation of muscarinic and nicotinic receptors (Gallo and Lawrk, 1991). Exposure of *Culex quinquefasciatus* larvae to 1.5 ppb of ivermectin leads to the development of ataxia and eventually death (Alves *et al.*, 2004). This result suggests a connection between ivermectin and GABA receptors (Freitas *et al.*, 1996).

Investigations of mosquito blood feeding and resting behaviour are of crucial importance for areas where epidemics occur and usually comprise the investigation of host-seeking and feeding behaviours on several vertebrate species, the measuring of endophagous/exophagous biting behaviour, endophilic/exophilic resting behaviour, and the mosquito's daily biting activity (recorded over 24 hours). Delatte *et al.* (2010) showed that *Ae. albopictus* prefer to feed (89% exophagic) and rest (87% exophilic) outdoors in contrast to *Ae. aegypti*, which is well-adapted to the highly urban

environments of tropical cities and frequently bite and rest indoors (Lambrechts *et al.* 2010).

Mosquitoes can be opportunist, feeding on a wide range of cold- and warm-blooded hosts, and thus can be involved in various vertebrate-virus transmission cycles, acting as a bridge vector for zoonotic viruses. Anthropophily (preference for humans) combined with multiple blood feeds during completion of one meal, increases the risks of spreading an arbovirus to the human population (Delatte *et al.*, 2010).

Blood-feeding behaviour can influence vector potential, depending on the vertebrate host groups with which the mosquito makes contact. If reservoir and amplification hosts (where the pathogen is surviving) are the primary focus of vector blood-feeding, the likelihood of pathogen acquisition by the vector increases. A mosquito species that can feed on wide range of hosts is a potentially dangerous bridge vector of zoonotic pathogens to humans (e.g. West Nile), but in contrast is likely to be less efficient as an epidemic vector of pathogens restricted to humans (e.g. dengue, chikungunya) (Lambrechts *et al.*, 2010).

In addition, the blood-feeding behaviour of a vector may influence the spatial distribution of a disease. The spatial distribution of a population of blood-sucking insects amongst its available vertebrate hosts has important epidemiological consequences for the transmission of vector-borne diseases. In fact, after daily survival rate, vector capacity is the one parameter most sensitive to changes in host preference (Dye and Hasibeder, 1986; Alonso *et al.*, 2003). Therefore, knowledge of the biological parameters that lead to

host choice can be highly relevant for the planning of vector and disease control (McCall and Kelly, 2002; Alonso *et al.*, 2003).

2.3 Habitats of Mosquito

Mosquitoes have diverse habitats that allow them to colonize different kinds of environments. The immature stages of mosquitoes are found in a variety of aquatic habitats like ponds, streams, ditches, swamps, marshes, temporary and permanent pools, rock holes, tree holes, crab holes, lake margins, plant containers (leaves, fruits, husks, tree holes, bamboo nodes), artificial containers (tires, tin cans, flower vases, bird feeders), and other habitats (Laird 1988; Rueda *et al.*, 2005, 2006).

The enormous importance of diverse habitats on the increasing populations of mosquitoes has been well recognized by aquatic ecologists and public health personnel. Furthermore, knowledge of larval habitats is important in determining vector control, as well as for disease prevention purposes. It is extremely necessary in designing effective vector control programs. The most practical way to reduce a local population of pestiferous mosquitoes is to eliminate their habitats as much as possible, particularly sources of standing water, such as old discarded tires, clogged gutters, and stumped tree holes. Larval habitats that are not possibly eliminated can be modified (e.g., cleaning clogged ditches, open water management in salt marshes). Appropriate methods can be applied such as using biological control agents (predatory fish, microbials), selected insecticides for permanent habitats (ponds, lakes), or other modification techniques to control breeding of environmental mosquitoes (Laird 1988; Rueda *et al.*, 2005, 2006).

2.4 Taxonomic Tree of *Culex quinquefasciatus*

Domain: Eukaryota

Kingdom: Metazoa

Phylum: Arthropoda

Subphylum: Uniramia

Class: Insecta

Order: Diptera

Family: *Culicidae*

Genus: *Culex*

Species: *Culex quinquefasciatus*

Culex quinquefasciatus belongs to the globally distributed *Culex pipiens* species complex which contains a number of related species, ecotypes or forms and hybrids that occur along geographical introgression zones on multiple continents (Farajollahi *et al.*, 2011). Thomas Say first described the species in 1823 from a specimen collected along the Mississippi River in the southern United States. Since that time a number of similar species around the world have been synonymized with *Culex quinquefasciatus*, most notably *Culex fatigans* Wiedemann (1828) from the Old World tropics (Stone, 1956; Belkin, 1977). Females of *Culex pipiens* and *Culex quinquefasciatus* are morphologically indistinguishable and hybrid zones for the two species are well documented. This led to the designation of *Culex quinquefasciatus* as a subspecies of *Culex pipiens* with the name *Culex pipiens quinquefasciatus* (Barr, 1957). More recent studies have documented distinct sympatric populations of *Culex pipiens* and *Culex quinquefasciatus* (Cornel *et al.*,

2003) and clear genetic differences (Smith and Fonseca, 2004) that have led, once again, to the elevation of *Culex quinquefasciatus* to species status. The other currently recognized species and subspecies in the species complex are *Culex pipiens pipiens*, *Culex pipiens pallens*, *Culex globocoxitus*, and *Culex australicus* (Farajollahi *et al.*, 2011). Two forms are recognized in *Culex pipiens pipiens*; form *pipiens* undergoes winter diapause and requires a blood meal for egg production while form *molestus* does not undergo diapause and is able to lay eggs without a primary blood meal (autogeny) (Fonseca *et al.*, 2006; Farajollahi *et al.*, 2011).

Following mating, the female seeks a blood meal. *Culex quinquefasciatus* are opportunistic feeders, feeding on mammals and/or birds throughout the night. Males survive only on sugar meals, while the female will take multiple blood meals. After a female mosquito digests the blood meal and the eggs develop, it finds a suitable place to lay her eggs, and the cycle begins again. A single female can lay up to five rafts of eggs in a lifetime (Gerberg *et al.*, 1994).

2.5 Mosquito Life Cycle

Following mating, the female seeks a blood meal. *Culex quinquefasciatus* are opportunistic feeders, feeding on mammals and/or birds throughout the night. Males survive only on sugar meals, while the female will take multiple blood meals. After a female mosquito digests the blood meal and the eggs develop, it finds a suitable place to lay her eggs, and the cycle begins again. A single female can lay up to five rafts of eggs in a lifetime (Gerberg *et al.*, 1994).

2.5.1 Adult mosquito

Adult *Culex quinquefasciatus* vary from 3.96 to 4.25 mm in length (Lima *et al.*, 2003). The mosquito is brown with the proboscis, thorax, wings and tarsi darker than the rest of the body. The head is light brown with the lightest portion in the center. The antennae and the proboscis are about the same length, but in some cases the antennae are slightly shorter than the proboscis. The flagellum has thirteen segments that have few to no scales (Sirivanakarn *et al.*, 1987). The scales of the thorax are narrow and curved. The abdomen has pale, narrow, rounded bands on the basal side of each tergite. The bands barely touch the basolateral spots taking on a half-moon shape (Darsie and Ward, 2005).

2.5.2 Egg

Common to the *Culex* genus, the eggs of *Culex quinquefasciatus* are laid in oval rafts loosely cemented together with 100 or more eggs in a raft, which will normally hatch 24 to 30 hours after being oviposited (Bates, 1949).

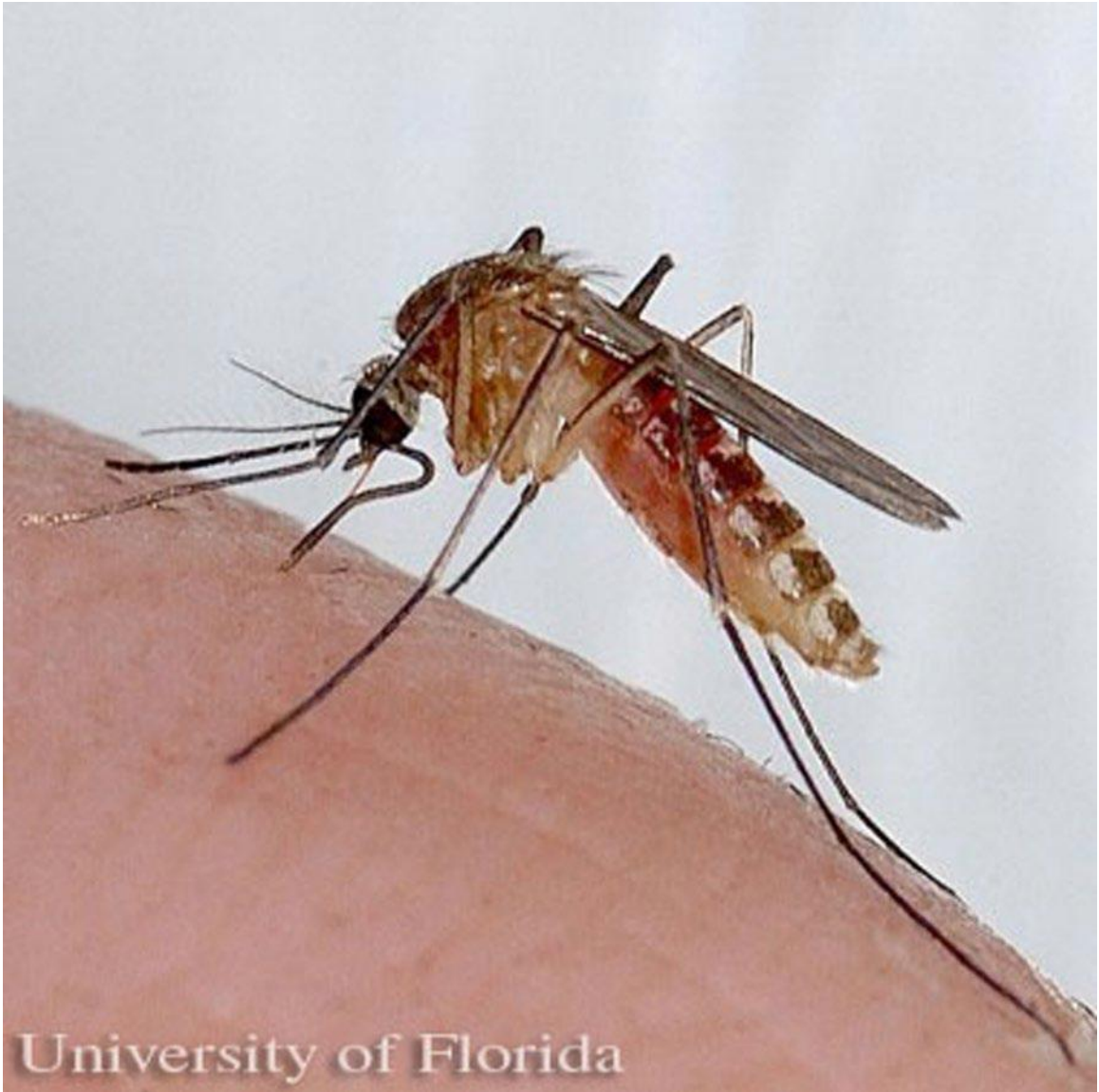


Figure 2.1: Adult female southern house mosquito, *Culex quinquefasciatus* Say.

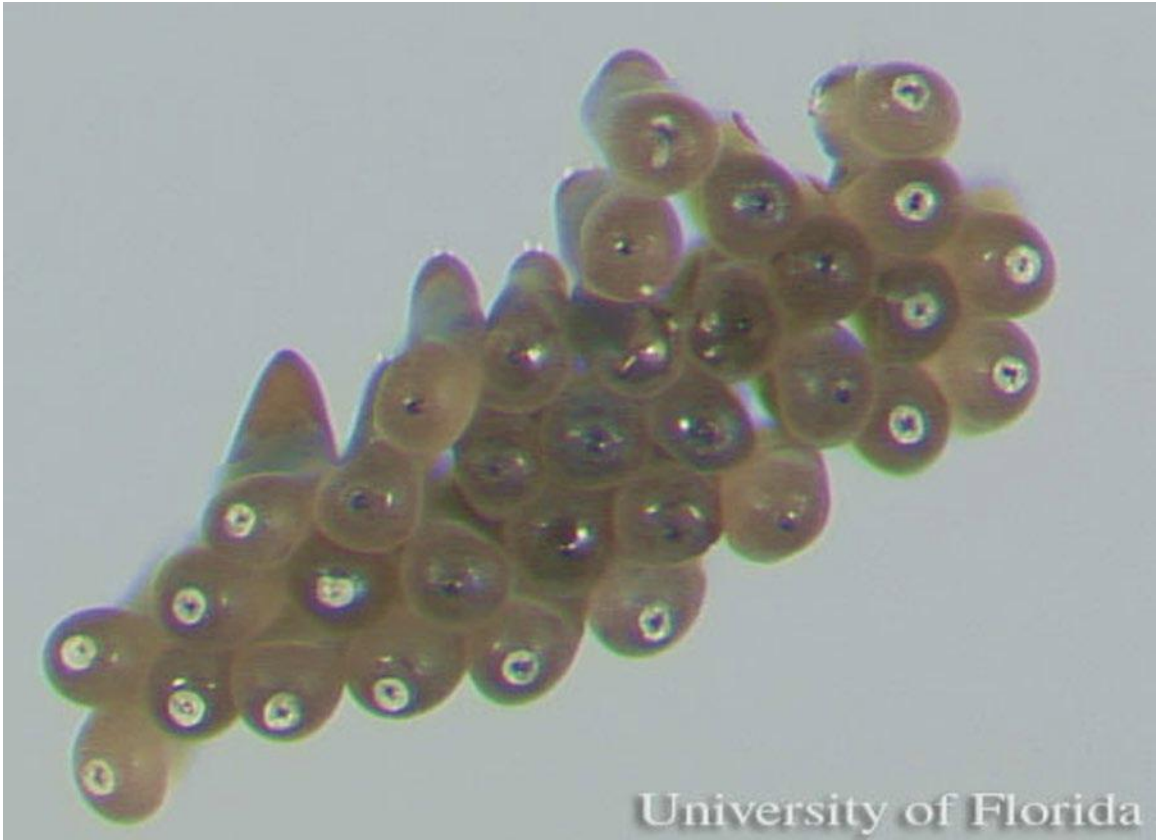


Figure 2.2: Eggs of the southern house mosquito, *Culex quinquefasciatus* Say.

2.5.3 Larvae

The larval head is short and stout, becoming darker toward 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. Each segment has a unique setae pattern (Sirivanakarn and White, 1978). The siphon is on the dorsal side of the abdomen, and in *Culex quinquefasciatus* the siphon is four times longer and it is wide with multiple setae tufts (Darsie and Morris, 2000). The saddle is barrel shaped and located on the ventral side of the abdomen with four long anal papillae protruding from the posterior end (Sirivanakarn and White, 1978).

2.5.4 Pupae

Similar to other mosquito species, *Culexquinquefasciatus* pupae are comma shaped and consists of a fused head and thorax (cephalothorax and an abdomen). The cephalothorax colour varies with habitat and darkens on the posterior side. The trumpet, which is used for breathing, is a tube that widens and becomes lighter in color as it extends away from the body. The abdomen has eight segments. The first four segments are the darkest, and the colour lightens towards the posterior. The paddle, at the apex of the abdomen, is translucent and robust with two small setae on the posterior end (Sirivanakarn and White, 1978).



Figure 2.3: Larva of the southern house mosquito, *Culex quinquefasciatus* Say.



Figure 2.4: Pupa of the southern house mosquito, *Culex quinquefasciatus* Say.

2.6 Species of Mosquito

The enormous importance of diverse habitats on the increasing populations of mosquitoes has been well recognized by aquatic ecologists and public health personnel. Furthermore, knowledge of larval habitats is important in determining vector control; as well as for disease prevention purposes (Rueda *et al.*, 2005, 2006).

2.6.1 Subfamily Toxorhynchitinae

The Toxorhynchitinae comprise a single genus, *Toxorhynchites*, which contains about 94 species that are mainly tropical, although a few species occur in North America, southeastern Russia and Japan. Adults are large (19mm long, 24mm wingspan) and colourful, being metallic bluish or greenish with black, white or red tufts of hair-like scales projecting from the posterior abdominal segments. Adults are easily recognized by having a proboscis that is curved backwards in both sexes and that is incapable of piercing the skin. Consequently, since neither sex can bite, they are of no medical importance (Service, 2012).

Their larvae are also large (12–18mm long), often dark reddish and, like those of the Culicinae, have a siphon. They are predaceous on larvae of other mosquitoes and on their own kind. They have occasionally been introduced into areas in the hope that their voracious larvae will help reduce the numbers of pest mosquitoes. Larvae are found mainly in container-habitats, such as tree holes and bamboo stumps, tin cans and water-storage pots (Service, 2012).

2.6.2 Subfamily Anophelinae

Of the three genera included in the subfamily Anophelinae only the genus *Anopheles* (about 477 species) is medically important, for instance as malaria vectors. The following characters serve to separate anophelines from culicine mosquitoes (Service, 2012).

2.6.2.1 *Anopheline eggs*

Eggs are laid singly on the water surface. In most species they are typically boat-shaped, and laterally have a pair of air-filled sacs called floats. Anopheline eggs are unable to withstand desiccation (Service, 2012).

2.6.2.2 *Anopheline larvae*

Larvae lack a siphon and lie parallel to the water surface, at an angle as are the culicines. They are surface-feeders and so spend most of their time at the water surface. Examination under a microscope shows that the abdomen has small, brown, sclerotized plates called tergal plates on the dorsal surface of abdominal segments 1–8; there may also be 1–3 small accessory plates behind a main tergal plate. In addition, most or all of these segments have a pair of well-developed palmate hairs, sometimes called float hairs. These abdominal palmate hairs and a single pair on the thorax come into contact with the water surface and aid in keeping larvae parallel to the surface. Laterally on each side of segment 8 (8 and 9 are combined) there is a sclerotized comb-like structure with teeth called the pecten. All these structures identify larvae as belonging to the genus *Anopheles* (Service, 2012).

2.6.2.3 *Anopheline pupae*

The respiratory trumpets of anopheline pupae are short and broad distally, thus appearing conical, whereas in most culicines the trumpets are narrower and more cylindrical. The most reliable character for identifying anopheline pupae is the presence of short, peg-like spines situated laterally near the distal margins of abdominal segments 2–7 or 3–7; in culicines there are no such spines (Service, 2012).

2.6.2.4 *Anopheline adults*

Adult *Anopheles* usually rest with their bodies at an angle to the surface that is with the proboscis and abdomen in a straight line, or ‘head down bottom up’. In some species they rest at almost right angles to the surface, whereas in others such as the Indian mosquito *Anopheles culicifacies* the angle is much smaller. This is a very useful character, allowing adults resting in houses and elsewhere to be readily identified as *Anopheles* (Service, 2012).

Most, but not all, *Anopheles* mosquitoes have the dark (usually black) and pale (usually white or yellowish) scales on the wing veins arranged in ‘blocks’ or specific areas forming a distinctive spotted pattern which differs according to species. A few species, however, such as the European *Anopheles claviger*, have the veins covered more or less uniformly with dark (often brown) scales. The most reliable way to distinguish between adult *Anopheles* and Culicinae is by examination of their heads (Service, 2012).

The first procedure is to determine the sex of the adults: female mosquitoes have non-plumose antennae whereas males have plumose antennae. If the adults are females and also *Anopheles*, then the palps will be about as long as the proboscis and usually lie

closely alongside it. The palps are usually blackish with broad or narrow rings of pale scales, especially on the apical half (Service, 2012).

In male *Anopheles* the palps are also about as long as the proboscis but are distinctly swollen at their ends and are said to be clubbed; they may also have rings of pale scales apically. Other differences are that in *Anopheles* there is only a single spermatheca in females, and in both sexes the middle lobe of the salivary glands is considerably shorter than the two outer lobes (Service, 2012).

2.6.3 Subfamily Culicinae

There are some 3053 species in the large subfamily Culicinae (belonging to 38 or 110 genera, depending on the classification used). The most important medically are the genera *Aedes*, *Culex*, *Mansonia*, *Haemagogus*, *Sabethes* and *Psorophora* (Service, 2012).

2.6.3.1 Culicine eggs

Culicine eggs never have floats. They are laid either as a number of single eggs (e.g. *Aedes*) or in the form of egg rafts that float on the water surface (e.g. *Culex* and *Coquillettidia*), or are deposited as sticky masses glued to the underside of floating vegetation, e.g. *Mansonia* (Service, 2012).

2.6.3.2 Culicine larvae

All culicine larvae possess a siphon, which may be long or short. They hang upside down at an angle from the water surface when they are taking air, except for *Mansonia* and *Coquillettidia* larvae, which insert their specialized siphons into aquatic plants and

remains submerged. There are no abdominal palmate hairs or tergal plates on culicine larvae (Service, 2012).

2.6.3.3 *Culicine pupae*

The length of the respiratory trumpets in culicine pupae is variable, but they are generally longer, more cylindrical and have narrower openings than in *Anopheles*. Abdominal segments 2–7 lack peg-like spines, although they have numerous setae (Service, 2012).

2.6.3.4 *Culicine adults*

Culicine adults rest with the thorax and abdomen more or less parallel to the surface. The scales covering the wing veins are commonly uniformly brown or black. Sometimes there are contrasting dark and pale scales, but they are not arranged in distinctive areas or 'blocks', as found in many *Anopheles* adults (Service, 2012).

The most reliable method for identifying the Culicinae is to examine their heads. In females (which have non-plumose antennae) the palps are shorter than the proboscis. In males (which have plumose antennae) the palps are about as long as the proboscis but are not swollen distally and hence, do not appear clubbed (Service, 2012).

However, the palps may be turned upwards distally, and in many species they are covered with long hairs so that superficially they can appear to be somewhat swollen apically, but more careful examination shows that the palps in male culicines are not clubbed as they are in *Anopheles* (Service, 2012).

Other differences separating the Culicinae from *Anopheles* are that in culicines there are two or three spermathecae in females, but just one in anophelines. Also, in culicines the

middle lobe of the salivary gland is about as long as the other two, whereas in anophelines it is shorter (Service, 2012).

2.7 Importance/Disease Vectors

Many members of the mosquito genera such as *Anopheles*, *Aedes*, *Culex*, and *Mansonia* that commonly bite humans and animals and are involved in the transmission of infectious diseases as principal, secondary or bridge vectors. They are a nuisance to humans and other animals. Although in several temperate countries mosquitoes may be of little or no importance in transmitting diseases to humans they can, nevertheless, cause considerable annoyance because of their troublesome bites (Service, 2012). In the presence of suitable climate and availability of infection sources, the likelihood of vector-borne infections increases with vector abundance. Transmission may also be possible with low or moderate vector densities if the vector population has a high competence (% of infectious females).

Changes in climate and environment mean that potential vectors and, with them, diseases that they can transmit can spread unnoticed to 'emerge' in new areas. Such spreading may not only be caused by active vector migration but can be due to their passive transport, for example by vehicles, trains and aircraft. Perhaps more importantly, the explosive increase and globalisation of international and intercontinental travels and trade increases the risk of accidental import of vectors and vertebrate reservoir hosts (i.e. vertebrates that carry a pathogen).

2.7.1 Malaria parasites

The four different species of human malaria parasite cause four different diseases *Plasmodium malariae* causes quartan malaria, *P. vivax* causes benign tertian malaria, *P. ovale* causes ovale malaria and *P. falciparum* causes the malignant tertian. Thus, malaria used collectively is a purely colloquial term. In any context demanding precision, it requires to be qualified according to the particular parasite concerned.

Plasmodium falciparum and its relative *P. reichenowi* of chimpanzees and gorillas are sufficiently distinct to be placed in a separate subgenus, *Laverania*. The other kinds of human malaria parasites are also all represented by related forms in apes and monkeys. Some of these are capable of establishment in man. Recent cases of natural and accidental infection of man with monkey parasites have been reported. Simian malaria is consequently now regarded as a zoonosis. By convention man is regarded as the intermediate, and the mosquito as the definitive host, since it is in the latter that fusion of gametes takes place.

2.7.2 Filarioid nematodes

Only two mosquito-borne species infecting man have been identified with certainty though, the existence of others is suspected. The species in question are *Wuchereria bancrofti* and *Brugia malayi*. Aside from the periodicity of the microfilariae, the life-histories of all forms of *Brugia* and *Wuchereria* are similar.

Infective larvae leave the proboscis on contact with the warm skin. A proportion penetrates the skin by way of the puncture made by the mouth-parts. No host other than man is available and consequently the time taken for maturation of the adults is unknown.

There are indications that it is longer than for *B. malayi* which takes about ten to fifteen weeks in cats. The adults are found mainly in lymph glands and lymphatics (Logan, 1993).

Wuchereriabancrofti and *B. malayi* occur in both periodic and sub-periodic forms. In the periodic forms the microfilariae concentrate in the vessels of the lung for most of the twenty-four hours, appearing in the peripheral circulation mainly during the middle part of the night. In the sub-periodic forms the nocturnal peak is suppressed and there is a more uniform distribution over the twenty-four hours, though still with a tendency to concentration in the peripheral blood when the vector is normally most active (Pampana, 1963).

The periodic form of *W. bancrofti* is distributed throughout the tropics, except in the Pacific area where it is replaced by the sub-periodic form. The latter has very much the appearance of a parasite introduced by man which has become adapted to new vectors. Its principal vectors are *Aedes* spp., notably members of the *Ae. scutellaris* complex. Those of the periodic form are various *Anopheles* spp. and *Cx. pipien fatigans*. Subperiodic *B. malayi*, unlike either form of *W. bancrofti*, is found in a wide variety of forest animals and can be easily transmitted to laboratory hosts. Very interestingly it remains subperiodic when transferred from man to domestic cats, assumes a nocturnal periodicity when passed from cats to monkeys and reverts to sub-periodicity when transferred from leaf monkey to cat.

2.7.3 Arboviruses

Of all the many mosquito-borne viruses less than a dozen have been studied, ecologically in any detail. The problems are formidable. To identify the vectors it is not sufficient simply to find infected mosquitoes. Not all species which acquire the virus are necessarily capable of transmitting it. Even if they are potential transmitters their contact with susceptible hosts may be insufficient for them to transmit with any regularity. A variety of vertebrate hosts may be found infected or with antibodies but not all of these will circulate virus in sufficient quantity to infect the vectors. During inclement season virus may die down and become difficult or impossible to locate. There may be annual reintroduction by migratory hosts. Finally, ecological conditions may vary so that what is true at one time or place may not be true at another. How, in the face of this, different arboviruses maintain their ecological specificity is a challenging question.

2.7.3.1 *Virus Encephalitides*

Mosquito-borne viruses capable of causing severe encephalitis in man include Western and Eastern equine, in Group A, St Louis, Japanese B and Murray Valley, in Group B, and California encephalitis virus in the California Group.

The last named differs from the others in being, it would seem, primarily a rodent virus, transmitted by species of *Aedes*. The first recorded human outbreak occurred as recently as 1964, in Indiana and neighbouring states, although the virus was discovered some fifteen years ago. The other viruses mentioned are essentially bird viruses associated either with marsh birds such as herons or with passerine birds feeding and nesting in marshy areas. Western equine encephalitis occurs mainly in the western United States though it has caused both human and equine outbreaks as far south as Argentina and

as far north as southern Canada. The principal vector, *Culex tarsalis*, has a distribution approximately coterminous with that of the virus in North America. It feeds chiefly on birds but also, readily, on mammals, including man, and on reptiles.

Among the other encephalitis viruses the best studied is Japanese B. The principal vector of this virus, in Japan, is *Culex tritaeniorhynchus*. The primary hosts are herons and ibises, in which there is an extensive build-up of virus in the spring. Domestic pigs become infected during the summer and human infections follow somewhat later. Horses become infected concurrently with, but much less often than, the pigs. In South-east Asia the situation is somewhat different. There all-year-round transmission is possible and pigs are thought to provide a permanent reservoir of virus.

2.7.3.2 Yellow Fever

Yellow fever differs ecologically in many respects from the encephalitis viruses. Principally, the possible hosts are primates. *Culex* species play no part, so far as is known, in its transmission. It is enzootic only within the tropics. Viraemia, in primates, is short lived and immunity, probably, lifelong. The relatively slow reproductive turnover in these hosts implies that the reservoir must be a highly dynamic one, with the virus continually shifting from one focus to another within its main area of distribution, which comprises the humid forested areas of West and Central Africa and northern South America.

In the drier parts of Africa, antibodies to yellow fever virus have been recovered from Galapagos Island as far east as the Kenya coast and the Islands of Zanzibar and Pemba. Curious, and at the present unexplained, relationships exist between immunity rates in

different types of host. Thus, in northern Uganda and much of Kenya the infection appears to be largely confined to Galapagos but in the southern Sudan antibodies are rare in Galapagos, common in man and monkeys. In Zambia, northern Lesotho and Malawi there is a high incidence of antibodies in man, no evidence of infection in monkeys. Although some aspects of the epidemiology of yellow fever seem to be fairly well understood, it is evident that there are still large gaps in the knowledge of the natural history of this most lethal of all arbovirus diseases. Closely related viruses are known only from Africa and this, coupled with the generally greater tolerance on the part of African primates, seems strongly to suggest a post-Columbian introduction of yellow fever into South America by man.

2.7.3.3 *Dengue*

Dengue syndrome can be produced by any one of the several viruses. These include Chikungunya and Onyongnyong in Group A and West Nile and the true dengue viruses, types 1, 2, 3 and 4 in group B. West Nile, which has been recorded from Israel, Egypt, East and South Africa and India, appears to resemble the encephalitis viruses in being primarily a virus of birds transmitted chiefly by *Culex*. The occurrence of true dengue viruses in non-human hosts has yet to be conclusively proven though antibodies to these or to a very close relative have been found in monkeys and other forest animals in Malaya. The disease was first recorded in epidemic form in India and Java in the late eighteenth century. During the present century there had been outbreaks in areas as widely separated as Greece, northern Queensland and Hawaii, though such outbreaks were relatively infrequent in South-east Asia until the recent appearance there of a new and lethal haemorrhagic form of the disease.

Haemorrhagic dengue was first recorded, unequivocally, in Manila in 1954 and in Bangkok in the same year, though there is some evidence for a small outbreak in Thailand as long ago as 1935. More recently outbreaks have been recorded from Singapore, Malaya, Laos, Vietnam and India. The disease appears to be becoming endemic over much of southern Asia. Unlike classical dengue, which is a painful and temporarily disabling, but not a lethal, disease the haemorrhagic form is quite highly lethal especially to children.

Chikungunya virus was first isolated in East Africa in 1952. It was subsequently recovered during the haemorrhagic dengue epidemic in Bangkok in 1958 and has more recently caused massive epidemics in a number of cities in India. It does not however, appear to produce haemorrhagic symptoms of a severe kind. The latter, so far as is known, are associated strictly with the classical dengue viruses.

Onyongnyong virus is closely related to Chikungunya and to the wild African Semliki Forest virus. It was first isolated from human cases in north-west Uganda in 1959. It subsequently spread southwards as far as Malawi, causing more than a million human cases. It is remarkable in being the only known human virus with anopheline mosquitoes as major vectors. The only vectors so far incriminated are *Anopheles gambiae* and *funestus*. Though still sub-lethal, Onyongnyong virus produces more severe symptoms than does Chikungunya. Semliki Forest is less pathogenic than either. Though shown to be transmissible by anophelines it has not been recovered from them in nature.

The involvement of man with all these viruses is clearly seen as the accompaniment to his progressive exploitation of his environment. Jungle yellow fever, in South America, is basically a disease of woodcutters, associated with the felling of trees and the bringing of infected Canopy mosquitoes to the forest floor in clearing. Rural yellow fever, in East Africa, is associated with the cultivation of broad-leaved food plants which serve as breeding places for the rural vector, *Aedes simpsoni*. Western equine encephalitis is associated with irrigated farmland, Japanese B with rice-cultivation and pig-breeding. With increasing urbanization have come ecologically new diseases urban dengue, urban yellow fever, haemorrhagic dengue, urban filariasis. Finally, in the very recent past, man has developed a new mode of exploitation, the large scale employment of synthetic insecticides. It is hard to believe that the emergence of Onyongnyong fever, the first anopheline-borne virus disease of man, has followed so closely, only by coincidence, on the use of these insecticides to alter the balance between anthropophilic and zoophilic *Anopheles*.

2.8 Prevention and Control

Various methods have been adopted in controlling mosquito species in general and *Culex quinquefasciatus* inclusive.

2.8.1 Prevention

Public health quarantine measures regarding human disease vectors aboard aircraft have been in place since the 1930s (Hughes, 1949). Disinsection by pyrethroid insecticides as an aerosol spray during flight and/or as residual surface treatment is recommended on all aircraft departing from countries with known disease vectors (Gratz *et al.*, 2000). Pre-embarkment treatments combining a rapid knockdown component (*d*-phenothrin) and a

residual component (permethrin) have been developed (WHO, 1995). In response to health concerns by airline cabin staff, aircraft disinfection is no longer a requirement of the United States (Sutton, 2007). Currently 24 countries (or island states) require disinsection by pyrethroid application for all or selected incoming flights (American Samoa, Australia, Barbados, China, Cook Islands, Cuba, Czech Republic, Ecuador (Galapagos), Fiji, France, Grenada, India, Jamaica, Kiribati, Madagascar, Mauritius, New Zealand, Panama, Seychelles, South Africa, Switzerland, Trinidad and Tobago, United Kingdom and Uruguay (Department of Transportation, 2012). Alternative, non-pesticide, methods of disinfection such as mechanical air curtains are being evaluated (Carlson *et al.*, 2006).

2.8.2 Public awareness

Public education is always a key component of successful mosquito control. Identification and removal or treatment of household- and agriculture-associated larval mosquito habitats can greatly reduce *Culex quinquefasciatus* abundance. This can be particularly significant when highly productive septic habitats are involved (Gimnig *et al.*, 2001).

2.8.3 Biological control

Culex quinquefasciatus has been experimentally eradicated from a village in Burma using cytoplasmic incompatibility (Laven, 1967) and in the Florida Keys (USA) using sterile males (Patterson *et al.*, 1970). Following these early successes, larger scale attempts made in India with sterile males, cytoplasmic incompatibility and genetic translocations failed due to immigration of wild males into the control area (Asman *et al.*, 1981).

2.8.3.1 Predators

Fish, especially the guppy *Poecilia reticulata*, have been used to successfully control *Culex quinquefasciatus* in a number of larval habitats throughout Africa, India and Southeast Asia (Subra, 1981; Chandra *et al.*, 2008). The cyclopoid copepod, *Macrocyclus albidus*, has also been used to control *Culex quinquefasciatus* larvae in open drainage ditches; however, copepods alone cannot eliminate this mosquito (Marten *et al.*, 2000).

2.8.3.2 Microbes (bacteria and viruses)

Various microsporidia, fungi and nematodes have also been tested for control of larval *Culex quinquefasciatus* with limited success in operational field applications (Subra, 1981; Guzman and Axtell, 1986). Perhaps the most successful agents for control of *Culex quinquefasciatus* have been the biopesticides or mosquitocidal toxins associated with the bacteria *Bacillus thuringiensis* serovar. *israelensis* (Bti) and *Lysinibacillus sphaericus* (*Bacillus sphaericus*). The efficacy of Bti against *Culex quinquefasciatus* is somewhat limited by higher concentrations of organic matter in the treated larval habitat. Unfortunately, resistance to both of these toxins has evolved rapidly in some field populations (Wirth *et al.*, 2000).

2.8.4 Chemical control

The simplest approach to control of *Culex quinquefasciatus* is source reduction of larval mosquito habitat. Most domestic habitats can be eliminated or modified to prevent access by mosquitoes while peridomestic habitats associated with agricultural practices may be modified or treated to reduce productivity (Hill and Connelly, 2013).

Crude petroleum oils were often used for larval mosquito control before the advent of the organochlorine insecticide, DDT. More refined oils are used today although they are generally expensive. For a decade or so following World War II, organochlorines were commonly used and quite effective against larval *Culex quinquefasciatus*. Following the evolution of resistance to organochlorines, organophosphates such as diazinon, fenthion, malathion, temephos and chlorpyrifos were used to control both larvae and adults (Subra, 1981). Today, many populations of *Culex quinquefasciatus* have evolved resistance to the most commonly used organophosphate, carbamate and pyrethroid insecticides (Jones *et al.*, 2012). Expandable polystyrene beads have been used in pit latrines and open septic systems to create a physical barrier to egg laying adults (Curtis *et al.*, 2002).

Today, most mosquito control programs have adopted an integrated pest management approach combining source reduction and biological control with an alternating use of biopesticides or conventional insecticides (Mulla, 2003). The concept of integrated pest management (IPM), the combination of different methods to limit pest infestations in crops, was first applied in the agricultural sector in the 1950s but has since evolved to broader definitions to include a framework for sustainability of the agricultural system (Morner *et al.*, 2002). In parallel, in the 1980s, the growing interest in vector control methods, other than the application of residual insecticides, led to the development of integrated vector control (IVC) strategies based on the principles of IPM. The IVC approach included: (i) personal protection; (ii) habitat management and source reduction; (iii) the use of insecticides both as larvicides and adulticides; (iv) an appreciation of the

possibilities of biological control by recognizing the role of fish in reducing larval numbers; and (v) training and education (WHO, 1983).

Nevertheless, problems have arisen with both IPM and IVC approaches. The acceptance and implementation of IPM has been slow, and IVC did not support sustainability or adequately consider economic impacts of interventions (Morner *et al.*, 2002). Following on from the lessons learned from IPM and IVC, the concept of integrated vector management (IVM) was developed to make optimal use of resources and tools for vector control (WHO, 2004).

However, it needs to be stressed that, for IVM, the term 'integrated' does not necessarily apply to a combination of more than one control tool, or the control of more than one disease, but to the incorporation of an effective intervention(s) into an integrated approach that includes the major components described above and disease mapping, monitoring and surveillance that can adapt control strategies to local environments. It has been recognized that, in the WHO Eastern Mediterranean Region, such efforts have been compromised by the spread of vector resistance to insecticides, weak management of insecticide deployment at the national level, and the increase in emerging and re-emerging vector-borne diseases (Mnzava *et al.*, 2011).

One important benefit of an IVM framework is the potential added value of a selected intervention impacting on more than one vector-borne disease. This is possible where the targeted vectors transmit more than one disease (e.g. *Anopheles* can transmit both malaria and lymphatic filariasis (LF), and *Stegomyia* mosquitoes can transmit LF and dengue) (WHO, 2011). Distributions of the targeted vectors and their associated diseases

can also overlap, for example, diarrhoea and LF can be controlled by targeting pit latrines and other human-waste-containing areas. The coordination of multidisease IVM could be facilitated by the use of improved technologies and data management systems (Eisen *et al.*, 2011). The challenges and constraints in the implementation of IVM have been fully discussed elsewhere (Mnzava *et al.*, 2011). In brief, at least for countries in the WHO Eastern Mediterranean Region (mainly comprising those of North Africa and the Middle East), these include:

- i. a lack of commitment to include IVM into a national health policy;
- ii. a reluctance to extend the mandate of existing disease-specific programmes to include other vector-borne diseases; and
- iii. complacency of countries with low vector-borne disease burdens (yet at risk of newly emerging vector-borne diseases) (Mnzava *et al.*, 2011).

Nevertheless, the WHO fully endorses an IVM strategy and is making efforts to ensure its implementation at the national level. For example, recent WHO publications have provided valuable information for countries and public health professionals wishing to plan and implement an IVM programme (WHO, 2012).

2.8.5 Botanical Control

Over the centuries scientists are exploring various methods to combat threats from mosquito-borne diseases and use of synthetic insecticides has been the major tool in mosquito control operations. However, the extensive and repeated use of synthetic organic insecticides such as organophosphates and organochlorines have led to disrupted natural biological control systems leading to resurgence and resistance in target species and destruction of nontarget beneficial fauna, in turn resulting in fostered environment and

human health concern(Tehri and Singh, 2015). The ill-effects of insecticide usage have thus, necessitated the need for research and development on environmentally safe, biodegradable and indigenous method for controlling mosquitoes(Tehri and Singh, 2015).

2.8.5.1 Botanicals as an essential component of IMM

Plants have co-evolved with insects that have equipped them with a plethora of chemical defense, which can, in turn be used against insects. Till date, more than 2000 plant species have been known to produce secondary metabolites of value in biological pest control programs and among these, products of some 344 species have been reported with significant activity against mosquitoes (Tehri and Singh, 2015).Larvicidal, adulticidal or repellent activities against different species of mosquitoes have been reported from members of the plant families Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae (Tehri and Singh, 2015).

The secondary metabolites present in plants constitute a defense system against insect/ pest attacks. The presence of compounds like phenolics, terpenoids and alkaloids present in plants, by acting as antifeedants, moulting hormones, oviposition deterrents, repellents, juvenile hormone mimics, growth inhibitors, antimoulting hormones as well as attractants, is held responsible for biological activity of plant extracts against target pest. Limonoids from Rutaceae particularly citrus have attracted greater apprehension due to their growth regulating activities (Tehri and Singh, 2015).

Citrus seeds are available in large quantity as waste products of the citrus industry from which citrus including limonin, nomilin, obacunone, epilimonol and limonin diosphenol can readily be extracted. Citrus limonoids have been found to work as feeding deterrents and also induce toxicity. It has been determined in structure-activity studies of limonin

that the furan ring and epoxide groups in the citrus limonoid structure are critical for the antifeedant activity of the limonoids. Limonoids lead to nutritional disruption inducing antifeedant effects that ultimately affect the egg laying process of insects (Tehri and Singh, 2015).

2.8.5.2 Mode of action of botanical pesticides

The mechanism of action of plant secondary metabolites on insect body was reviewed by Rattan, 2010. Several physiological disruptions, such as inhibition of acetylcholinesterase synthesis by essential oils, GABA-gated Chloride channel (by thymol), sodium and potassium ion disrupting exchange by pyrethrin and inhibition of cellular respiration by rotenone are reported in insects subjected to botanical treatment (Tehri and Singh, 2015). Other mechanisms include the blockage of calcium channels by ryanodine, of nerve cell membrane action by sabadilla of octopamine receptors by thymol, hormonal balance disruption, mitotic poisoning by azadirachtin, disruption of the molecular events of morphogenesis and alteration in the behavior and memory of cholinergic system by essential oil.

Of all these mechanisms, the inhibition of acetylcholinesterase activity (AChE) is the most important as it is the key enzyme responsible for terminating the nerve impulse transmission through synaptic pathway; AChE has now been observed to be organophosphorus and carbamate resistant, and it is well-known that the resistance in AChE is one of the main resistance mechanisms in insect pests (Tehri and Singh, 2015).

The observed histopathological effects of the ethanolic extract of *M.azedarach* on the midgut of *Culex quinquefasciatus* larvae were in agreement with the results obtained by

Hamouda *et al.*, (1996), Hussin and Shoukry(1997) and Assar and El- Sobky (2003) on *Culex pipiens*. Hamouda *et al.*, (1996)stated thatthe midgut of *Culex pipiens* treated with *Artemisia judaica* was affected,the epithelial layer was vacuolated, swollen cells, massesof cellular material appeared in the lumen and finally theepithelium lost their normal appearance(Al-Mehmadi and Al-Khalaf, 2010).Also, Hamouda *et al.* (1996)found thatlarvae treated with *Anagallis arvensis* showed a rupture of thecell wall and destruction of the peritrophic membrane. Assar and El-Sobky (2003)observed that the water extract of*Eichhornia crassipes*, revealed drastic effect on larval midgut asthe brush border and some of the epithelial cells were apicallydegenerated after 48 h and after 72 h, most of the epithelialcells completely degenerated and vacuolated.

The medicinal plant active ingredients have major targets of action that include, enzymes, hormones, protein kinases and other targets yet to be identified that play important roles in cellular functions. Azadirachtin and neem products exhibit various modes of action against insects such as anti-feedant, insect growth regulator (IGR), fecundity suppression and sterilization, oviposition repellency or attraction, affecting biological fitness and blocking development of vector-borne pathogens (Fallatah, 2010).

These bioactivities of neemproducts have been investigated on manyimportant disease vectors includingmosquitoes, flies and triatomine bugs.Azadirachtin mode of action as an IGR isblocking synthesis and release of moultinghormones (ecdysteroids) from the prothoracicgland. This causes incomplete ecdysis ofimmature stages and sterility in adults(Fallatah, 2010). Seedof Meliaceae trees as *Azadirachta* and *Meliaspp.*, contain azadirachtin, salannins andvolkensin triterpenoids (meliatoxins ormelicarpins) and

melarietenins that function as anti-feedants and stomach poisons against chewing insects such as desert locusts and mosquito larvae (Fallatah, 2010). Seeds of many plant species contain protective compounds that are reactive and toxic to most other organisms, including insecticides and repellents of insect pests as herbivores (Fallatah, 2010).

In a study conducted under laboratory conditions to monitor behavioral changes, it was revealed that *Aedes aegypti* larvae exhibited a natural behaviour with the siphon pointed up through the water surface and head hung down immediately after exposure to ethanol-extract of celery, *Apium graveolens* seeds in test solution (Tehri and Singh, 2015). All the larvae were found restless and performed aggressive self-biting to their anal papillae with their mouth parts and formed a ring shape (head to siphon) between 5 to 10 minutes after treatment with the extract at a concentration ranged between 200-500 ppm. Fifteen to thirty minutes after treatment, most of the larvae were found to be irritated showing erratic movements, wriggling up and down erratically and violently (Tehri and Singh, 2015).

This restless behavioral pattern persisted till the larval movement slowed down and the larvae failed to reach the water surface. At four hours after treatment high level larval knockdown was clearly seen onto the bottom of the glass beaker as a result of chronic paralysis. Knockdown rate varied in a concentration dependent manner and was found to gradually increase upon increasing the extract concentration from 100 to 500 ppm. The theoretical KD_{50} value was obtained as 238.15 ppm. This alteration in larval behaviour suggests that ethanol extract derived from the seed of celery could act as cytolytic, affecting the neuro-muscular coordination in the chemical synapse conduction, thus leading to aggressive self-biting, trembling movement, spinning and uncoordinated activity and paralysis (Tehri and Singh, 2015).

These symptoms were observed to be similar to those caused by synthetic nerve poisons, i.e. excitation, convulsions, paralysis and death. Discharge of electrolytes has also been observed from the anal region of treated mosquitoes as a result of the photo enhanced cytotoxic activity of the extract. Similar behavioral observations have been reported from the same plant by Choochote *et al.* (2004). Apart from contributing to the elucidation of mode of action of insecticides, observation of the poisoning symptoms of insecticides is also of practical importance for insect control. Extensively damaged and shrunken cuticle of the anal papillae in *Aedes* larvae was reported by Chaithong *et al.* (2006) when treated with pepper extract.

Alpha-terthienyl when introduced into the water medium containing mosquito larvae, entered into the body and subsequently caused halide leakage, releasing all the electrolytes into the medium and leading to death of the larvae (Tehri and Singh, 2015). An increase in the superoxide dismutase activity from 1st instar to 4th instar *Aedes* larval stage was observed by Nivsarkar *et al.* (1991). This increase seems to be a protective mechanism against hazardous oxygen derivatives generated by the action of the phototoxin alpha-terthienyl superoxide dismutase found in the entire gill, except in the tracheal network.

Further studies revealed severe morphological disruption of anal papillae in dead *Culex quinquefasciatus* larvae. Damaged anal papillae, with a shrunken cuticle border and destroyed surface with loss of ridge-like reticulum were observed under light and scanning electron microscopy after treatment with ethanolic extract of *Kaempferia galanga* (Tehri and Singh, 2015).

Similar distinct features of alteration were reported by Green *et al.* such as highly swollen anal papillae of *Ae. aegypti* larvae after treatment with oil of *Tagetes minuta*. The root extract of *Derris urucu* has been reported to affect the peritrophic matrix structure of *Ae. aegypti* larvae causing damage to the midgut epithelium. Midgut is the main site of digestion and absorption in insects (Tehri and Singh, 2015).

The non-cellular membranous structure, “peritrophic membrane” lining the midgut lumen, protects the mid gut cells from toxic substances and pathogens entering the midgut through food. Gut disruption by the activity of phototoxic Alpha-terthienyl was also observed earlier in other insects (Tehri and Singh, 2015). Botanical extracts are believed to contain growth regulatory compounds which possibly generate hormonal imbalance in the insects’ body. Formation of pupal-adult intermediates and ecdysal failure seem to be important cause of mortality. Treatment of immature mosquitoes with juvenile hormone (JH) analogues and chitin synthesis inhibitors also reported similar abnormalities (Tehri and Singh, 2015).

The natural plant products detrimentally affect insect growth and development. Ecdysis, shedding of old cuticle of insects, is under the influence of the hormone ecdysone. When the active plant compounds enter into the body of the insect, they may die due to abnormal regulation of hormone-mediated cell or organ development. Death may also occur either from a prolonged exposure at the developmental stage to other mortality factors or from an abnormal termination of a developmental stage itself. In particular, there often appears to be an incomplete extrication of the pupal stage from the larval cuticle,

while several adults are stuck to the chitin inner lining of the puparium (Tehri and Singh, 2015).

2.9 Selected Ethnomedicinal Plants and their uses

Nature has made plants useful throughout the existence of man. Man uses plants as food, clothing, fuel, shelter and the most useful necessity of life which is the maintenance and management of different diseases. Man suffers many diseases and God has provided the cure through the use of plant's roots, seeds, leaves, flowers, berries, or bark for medicinal purposes. Today, most pharmaceutical drugs are derived from excellent ingredients in medicinal plants. In folk medicine, many natural raw drugs have the potential to treat many diseases and disorders (Duke and Wain, 1981).

2.9.1 Annona senegalensis

Annona senegalensis belongs to the family Annonaceae, generally known as 'African custard-apple' and usually known as Gwándàn dààjì (Hausa), dukuu-hi (Fulani). The plant is widespread throughout savannah or sub-tropical regions of Nigeria and used in the treatment of diseases. African custard-apple plant is a wild growing shrub or small tree up to 7 meters or more, but this plant is not resilient in nature. This plant was cultivated for leaves, fruits, flower, bark, and stem for medicinal purpose. Leaves are alternate, simple, oblong to oval to almost ovoid, 6- 185 x 25- 120 mm in size. The stem are different in their natural colour as is grey and smooth to coarsely in older trees. The undeveloped branches are with yellow hairs which are lost during development. The flowers are up to 35 mm in diameter, on stalk up to 30 mm long, directly above the leaf axils. The fruit is formed from several fused, freshly and ovate carpels about 45 mm in

diameter. At early development, it is dark green ripening to yellow and finally to orange during the developmental stage of life. It has a curved inner whorl over the stamens and ovary and several stamen (Coates, 2002).

2.10.1.1 Uses of Annona senegalensis

The fruit obtained from this multipurpose plant is widely used locally in the treatment of two commonly energy deficiency syndrome known as kwashiorkor and marasmus. Dalziel (1937) reported about the plant to be of great medicinal value and its uses in native medicine which include headache and body ache (Arnold and Gulumian, 1984; Chhabra *et al.*, 1987), eyelid swelling (Klaus and Adala, 1994). The stem bark of *A. senegalensis* is used by local populations all over Africa in treating guinea worms infection, diarrhea and especially in northern Nigeria, gastroenteritis, snake bites, toothache, respiratory infections and malaria (Obiet *et al.*, 2003; Peters, 1965). Awa *et al.* (2012) reported the use of leaves in the treatment of pneumonia, and as a stimulant to improve health. A decoction from the roots is used to stop chest colds, venereal diseases, stomach ache and dizziness (Awa *et al.*, 2012).

2.10.2 Hibiscus asper

Among plant species, *Hibiscus asper* Hook.f. (Malvaceae) is an important medicinal plant widely distributed throughout tropical Africa and Madagascar (Sunil *et al.*, 2009). This species belongs to the genus *Hibiscus* represented by 250 species and characterized by the presence of biological active compounds like flavonoids, phenolic acids, and polysaccharides (Zhang, 2004). In the western region of the Africa, the plant is widely used by the traditional practitioners for the treatment of inflammation, anemia, jaundice,

leucorrhoea, poison antidote, depression and dysmenorrhea (Vasudeva and Sharma, 2008).

2.10.2.1 *Uses of Hibiscus asper*

In the western region of Africa, this plant is widely used by traditional practitioners for the treatment of inflammation, anemia, jaundice, leucorrhoea, poison antidote, depression and dysmenorrhea (Schippers and Bosch, 2004; Foyet *et al.*, 2011). In the western region of Cameroon, the leaves are highly recommended by traditional practitioners for the treatment of abscesses, urethritis and joint pain, but are also used as a potent sedative, tonic and restorative. It is also used to treat male infertility and skin infection (Burkill, 1985). In veterinary medicine, *H. asper* is used against the cutaneous infections of the domestic animals, as well as an antiparasitic drug (Schippers and Bosch, 2004). In our previous work, we demonstrated that methanolic extract of *H. asper* leaves extract have antioxidant effects and improves spatial memory deficits in the 6-OHDA lesion rodent model of Parkinson's disease (Foyet *et al.*, 2011). The present study was prompted by the claim of some Cameroonian traditional health practitioners that decoctions or infusions of *H. asper* leaves are effective remedies for the treatment or control of painful, arthritic and other inflammatory conditions.

2.10.3 *Hibiscus esculentus*

Okra, *Hibiscus esculentus* L. (Syn. *Abelmoschus esculentus*) (EEO) is one of the members of the Malvaceae family. It is known by many names such as Lady's finger, Bamyah and Bhindi; which is eaten raw as well as cooked. The geographical origin of okra is disputed, with supporters of South Asian, Ethiopian and West African origins. Supporters of a South Asian origin point to the presence of its proposed parents in that region. Supporters

of West African are of the view that greater diversity of okra are found in that region (Mayset *et al.*, 1990). The species is a perennial, often cultivated as an annual in temperate climates, and often grows to around 2 m tall. It is related to such species as cotton, cocoa, and hibiscus. The leaves are 10–20 cm long and broad, palmately lobed with 5–7 lobes. The flowers are 4–8 cm in diameter, with five white to yellow petals, often with a red or purple spot at the base of each petal. The fruit is a capsule up to 18 cm long with pentagonal cross-section, containing numerous seeds (De Rosa *et al.*, 2010).

2.10.3.1 Uses of Hibiscus esculentus

Egyptians believed that eating okra prevents the development of urinary calculi (Kamal, 1975). In folkloric practice fresh tender pods were given to cure constipation, leucorrhea, spermatorrhea, diabetes and jaundice (Chopra *et al.*, 1956). Mucilage is also used as soothing emollient medicine in the treatment of diarrhea, dysentery and gastric ulcer. A cupful of mucilage mixed with a ripe banana is given as a tonic-food during the treatment of colitis, cystitis, hepatitis and jaundice (Chopra *et al.*, 1956; Aman, 1969). A gastroprotective effect of the methanolic extract of okra in ethanol-induced gastric ulcer in rats was reported (Gurbuz *et al.*, 2003). Recently, some major antioxidant quercetin derivatives were identified and isolated from okra (Shui and Peng, 2004). Fatty and amino acids and minerals, especially zinc contents (Hirose *et al.*, 2003), and lepidimoides have been isolated from okra mucilage, which has multiple functions in the growth and development of plants (Cook *et al.*, 2000). Based on its folkloric use and reported antioxidant constituents, Alqasoumi (2012) also evaluated the protective effect of the ethanolic extract of okra (EEO) against carbon tetrachloride-induced hepatocellular injury in rats.

2.10.4 *Hibiscus cannabinus* (Kenaf)

It belongs to malvaceae family and is woody to herbaceous annual, mostly unbranched, fast-growing, with prickly stems, up to 4.2 m tall; leaves alternate, long-petiolate, shallowly to deeply parted, with 3–7 toothed lobes; flowers solitary, large to 10 cm in diameter, short-stalked, axillary, yellow with purple centers; sepals 5, petals 5; stamens numerous, connate; ovary superior; fruit a many-seeded, hairy capsule about 1 cm long; seeds brown, glabrous, wedge-shaped, 5 mm long, 3 mm wide, weight 25g/1000. Root a deep-penetrating taproot with deep-seated laterals (Reed, 1976). Probably it is native to Africa, East Indies, Asia, or Australia, now naturalized in Africa and Asia, generally cultivated in India, and introduced in India, Indochina, Taiwan, Indonesia, and North and South America. Its distribution is between 45°N and 30°S (Orwa *et al.*, 2009).

2.10.4.1 *Uses of Hibiscus cannabinus*

Kenaf is cultivated for its bast fibers which resemble and substitute for jute fibers. Fiber strands, 1.5–3 m long, are used for rope, cordage, canvas, sacking, carpet backing, and fishing nets. It is cultivated secondarily for the seeds which contain about 20% oil, used for: salad, cooking, and lubricant oils. Oil is also used in the manufacture of soap, linoleum, paints and varnishes, and for illumination. In 1968, Florida used 600 ha growing kenaf for bean poles (Whitely, 1981). Recently the pulp has been used in paper-making. Leaves are used as a potherb. A concentrated food for cattle in the form of seed-cake comes from the residue after oil extraction. Africans use soot from the stems as a black pigment. They also use a piece of the stem as a base for drilling fire (Orwa *et al.*, 2009).

Reported to be anodyne, aperitif, aphrodisiac, fattening, purgative, and stomachic, kenaf is a folk remedy for bilious conditions, bruises, fever, and puerperium (Duke and Wain, 1981). Powdered leaves are applied to guinea worms in Africa. Africans use peelings from the stems for anemia, fatigue, lassitude, etc. In Gambia, the leaf infusion is used for coughs. Ayurvedics use the leaves for dysentery and bilious, blood and throat disorders. Seeds are applied externally to aches and bruises. Medicinally, juice of the flowers with sugar and black pepper is used in biliousness with acidity. Seeds are considered aphrodisiac and fattening (Orwa *et al.*, 2009).

2.10.5 *Hibiscus lunariifolius* Willd

Hibiscus lunariifolius is an annual herb from the Old World tropics. Its flowers lack epicalyces, and have white or yellow petals (Exell and Meeuse, 1961). Erect shrubs, sparsely tomentose. Leaves; distant, alternate, 10 cm across, broadly ovate, cordate at base, acuminate at apex, crenate, densely softly tomentose below. Flowers in subterminal and terminal panicles; pedicels 5-6 cm long, jointed towards the end; sepals ovate, obtuse, cuspidate, connate at base into a short campanulate tube, pubescent outside; petals 2 x 0.9 cm, yellow, obovate, obtuse; stamens many; filaments combined into a tube. The nature of Ovary is seven-celled with many ovules in each; styles as many as the carpels; stigma terminal. Fruit a capsule, 15 x 15 mm, truncate at apex; mericarps beaked, seeds many, reniform, 2.5 mm across, glabrous, grey (Exell and Meeuse, 1961).

2.10.5.1 Uses of *Hibiscus lunariifolius*

It has various uses which include the following:

Landscaping

Paper

Beverage (*Hibiscus* tea)

Food

2.10.6 *Hibiscus sabdariffa*

Hibiscus sabdariffa ruber is an annual, erect, bushy, herbaceous subshrub that can grow up to 8 ft (2.4 m) tall, with smooth or nearly smooth, cylindrical, typically red stems. The leaves are alternate, 3 to 5 in (7.5–12.5 cm) long, green with reddish veins and long or short petioles. The leaves of young seedlings and upper leaves of older plants are simple; lower leaves are deeply 3 to 5 or even 7 lobed; the margins are toothed. Flowers, borne singly in the leaf axils, are up to 5 in (12.5 cm) wide, yellow or buff with a rose or maroon eye, and turn pink as they wither at the end of the day. At this time, the typically red calyx, consisting of 5 large sepals with a collar (epicalyx) of 8 to 12 slim, pointed bracts (or bracteoles) around the base, begins to enlarge, becomes fleshy, crisp but juicy, 1 1/4 to 2 1/4 in (3.2–5.7 cm) long and fully encloses the velvety capsule, 1/2 to 3/4 in (1.25–2 cm) long, which is green when immature, 5-valved, with each valve containing 3 to 4 kidney-shaped, light-brown seeds, 1/8 to 3/16 in (3–5 mm) long and minutely downy. The capsule turns brown and splits open when mature and dry. The calyx, stems and leaves are acid and closely resemble the cranberry (*Vaccinium* spp.) in flavour (Salama and Ibrahim, 1979). *Hibiscus sabdariffa* belongs to the Malvaceae family, it is the plant where its leaves and stems are commonly referred to as *Hibiscus* tea (whereas other species of the *Hibiscus* family such as [*Hibiscus macranthus*](#) are not commonly used as teas) and sometimes is referred to the colloquial name of Roselle or Sour Tea (Salah *et*

al., 2002). *Hibiscus Sabdariffa* also called Roselle or Sour Tea, is a tea where the usually dark colored flowers are used to brew. It appears to inhibit carbohydrate absorption to a degree and appears to be effective in reducing blood pressure (Ademiluyi and Oboh, 2012).

2.10.6.1 Uses of Hibiscus sabdariffa

Hibiscus sabdariffa (Roselle) is a supplemental herb that is derived from the plant's calyces, which are the collection of sepals separating the blooming flower from the stem. The calyces have traditionally been steeped into tea where the anthocyanins (red-blue pigmentation) are steeped into the water and drunk for medicinal purposes (Sayago-Ayerdi *et al.*, 2007).

Although it has a variety of claims medicinally, it appears to have evidence to support its role in reducing blood pressure in persons with elevated blood pressure. It may be this through ACE inhibitory potential (although this is admittedly weak) or benefitting the endothelium via Nitric Oxide related mechanisms (appears to be in better accordance with the amount of anthocyanins that reach the blood). Reductions in both diastolic and systolic blood pressure have been noted, and for the most part appear to be reliable in presence although not so much in magnitude of benefit which as blood pressure is reliably reduced, but the degree of reduction seems to vary (Sayago-Ayerdi *et al.*, 2007).

In regards to diabetes and blood glucose control, Roselle appears to have limited evidence to support these claims but the evidence is so far in support. Mechanisms are not known, and the remarkable potency in animal studies seems to be markedly less in the limited human interventions looking at it. Roselle does appear to weakly inhibit carbohydrate absorption enzymes, yet is synergistic with *Morus alba* (White Mulberry) in

doing so; a tea made of White Mulberries and Roselle, although currently not supported *in vivo*, is possibly an effective carbohydrate absorption inhibitory tea (Sarr *et al.*, 2009). The interactions of Roselle and weight loss are not too clear-cut, and it seems to be highly intertwined with studies on Roselle toxicity; Roselle is known to be toxic in higher doses, and weight loss more often than not precedes chronic toxicity. For studies that note weight loss without toxicity, it seems to be related to reduce food intake in rats and mice rather than direct fat burning effects (Salah *et al.*, 2002).

2.10.7 *Urena lobata*

Urena lobata is a plant of the family Malvaceae; its fibre is one of the bast fibre group. The plant, probably is of Old World origin, grows wild in tropical and subtropical areas throughout the world. The plant, an herbaceous perennial, is usually many-branched in the wild state and grows about 1 to 2 metres (3 to 7 feet) high. Cultivated plants, densely sown, reach 3 to 4.5 metres (10 to 15 feet) in height, with branches and leaves mainly concentrated near the top. The leaves vary in size and shape but are usually somewhat round, with 3 to 7 lobes and serrated edges. The flowers, growing singly in the axil of the leaf, have five petals that are usually pink. The small seeds have hooklike appendages and are produced in the greatest quantities by uncultivated plants (Onoagbe *et al.*, 2010). The name *Urena* apparently derives from the name given to the plant on India's Malabar Coast. *Urena* grows best in hot, humid climates, with direct sunlight and rich, well-drained soil. It is found wild in the tropical and temperate zones of North and South America and in Asia, Indonesia, the Philippines, and Africa. Cultivated crops, usually grown as annuals, are found mainly in the Congo Basin and Central Africa, with smaller plantings in Brazil, India, and Madagascar. Harvesting when the plants are in full flower,

yields fibre of high quality. The plants' stalks are cut by hand, above the woody plant base. After the stalks are subjected to a retting operation, the fibres are removed by hand (Omonkhua and Onoagbe, 2011).

2.10.7.1 Uses of Urena lobata

Urena has long been used for its fibre in Brazil, but it has been slow in achieving importance as a cultivated fibre crop and is still considered a troublesome weed in some countries. Commercial cultivation of the plant began in the Belgian Congo in the 1920s and in Central Africa in the 1930s. *Urena* fibre is lustrous and creamy white or pale yellow in colour. The fibre strands are about 1 metre (3.3 feet) long. *Urena* fibre is fine, soft, and flexible and is readily dyed. Used much like jute, which it resembles in appearance and strength, *Urena* is made into cordage, burlap (hessian), sacking fabrics, and carpeting materials and is often blended with jute or other fibres (Ramaiyan *et al.*, 2012). Other uses include the following:

- i. Antioxidant

Effects of *Irvingia grandifolia*, *Urena lobata* and *Carica papaya* on the Oxidative Status of Normal Rabbits: With recognized therapeutic effects, the plants were studied for toxic side-effects. Results showed no evidence of oxidative damage on liver and pancreatic MDA levels on rabbits, and even seemed to provide protection against lipid peroxidation (Constant *et al.*, 2012).

- ii. Phytochemical / Antioxidant / Antimicrobial

The study of *Urena lobata* leaf extract isolated 3 compounds: kaempferol, quercetin and tiliroside which showed strong antimicrobial activity against *E. coli*, *Bacillus subtilis* and

Klebsiella pneumoniae. The study supports the traditional use of the plant for treatment of infectious diseases (Mshelia *et al.*, 2013).

iii. Antidiarrheal/Seed Extract

A study reports the antidiarrheal potential of seed extracts of *L. dealbata* and *Urena lobata* used in the traditional medicine by the Naga tribes of India. Both plants showed significant inhibitory activity against castor oil-induced diarrhea and PGE₂-induced intrafluid accumulation. Both showed significant reduction in gastrointestinal motility with no signs of toxicity. Results help explain its traditional use as an antidiarrheal agent (Ramaiyan *et al.*, 2012).

iv. Antibacterial activity

A study of the methanol extract of *Urena lobata* showed a broad spectrum of antibacterial activity. Also a Comparative study of methanolic extract of the root and a standard herbal formulation showed antibacterial activity (Mazumder *et al.*, 2002).

v. Immunomodulatory

vi. Furocoumarin / Imperatorin

Studies have previously yielded mangiferin and quercetin from the aerial parts of the plant. This study isolated imperatorin, a furocoumarin, from the roots (Kusumba, 1998).

vii. Anti-Diabetic/Hypolipidemic

Study of aqueous extracts of *Urena lobata* (roots and leaves) in STZ-induced diabetic rats showed recognizable hypoglycemic/anti-diabetic and anti-hyperlipidemic effects (Onoagbe *et al.*, 2010).

viii. Hypoglycemic / Long-Term Effects of Root Extract

Study in rabbits showed *Urenalobata* aqueous extract of roots significant reduced body weight and fasting glucose. It exerted an initial toxic effect on hepatocytes and also caused bile obstruction. However, the effects were not severe and not sustained. A reduction in dose, frequency, and duration of administration may reduce the side effects observed in the study (Rinku *et al.*, 2010).

ix. Antioxidant/Roots

Study evaluated the methanolic extract of roots of *S. retusa*, *T. rhomboidea*, and *Urenalobata* for antioxidant activity. The extracts were found to inhibit lipid peroxidation scavenge hydroxyl and superoxide radicals *invitro*. Results showed all three possessed significant antioxidant activity (Lissy *et al.*, 2006)

x. Antifertility/Spermatogenesis Effect

Study evaluated *E. axillare* leaves and *Urena lobata* roots for antifertility activity in adult male Wistar albino rats. Results showed *E. axillare* and *Urena lobata* reversibly inhibited spermatogenesis and steroidogenesis indicating reversible antifertility activity (Ramaiyan *et al.*, 2010).

xi. Antioxidant/Cytotoxic/Leaves

Study evaluated a methanolic extract of leaves for antioxidant and cytotoxic potentials. Extract showed potent antioxidant activity with effective scavenging of free radicals and potent cytotoxic activity in the brine shrimp lethality assay (Sekendar *et al.*, 2013).

xii. Liver Effect/Toxicity Study

Study evaluated the effects of aqueous extract of root on liver of adult Wistar rats. Results showed that biochemical and morphological organization of the liver can be

significantly altered with continued and increased use of the extract (Mshelia *et al.*, 2013).

xiii. Antihyperglycemic/Antinociceptive/Leaves

Study of methanolic extract of leaves showed antihyperglycemic and antinociceptive effects. Alkaloids, flavonoids, saponins and tannins present in the methanolic extract may be responsible for the antinociceptive effect (Hasibul *et al.*, 2015).

xiv. Sperm Abnormality Effects

In a pilot toxicity study in albino rats, *Urenalobata* caused a significant increase ($p < 0.05$) in headless tail sperm cell abnormality, a primary sperm abnormality caused by a disruption in the course of spermatogenesis (Oladele and Abatan, 2010).

xv. Wound Healing

Study evaluated a methanolic extract for wound healing activity in albino rats. Results showed significant wound healing activity in excision, incision, burn, and dead space wound models, comparable to the Povidone-Iodine formulation. (Rinku *et al.*, 2013)

xvi. Antidiarrheal/Leaves

Study evaluated the antidiarrheal effects of *L. dealbata* seed extract and *U. lobata* leaf extract in castor oil induced diarrhea and PGE₂-induced intrafluid accumulation in murine models. Both extracts showed significant reduction in gastrointestinal motility in the charcoal meal test. Acute toxicity tests showed no sign of toxicity in the animals (Arun and Vareishang, 2007).

2.10.8 *Vernonia amygdalina*

Bitter leaf (*Vernonia amygdalina*) is a vegetable used for preparing the popular [Bitter leaf soup](#). It is also known as Onugbu, Shiwaka and Ewuro by the Igbos, Hausas and Yorubas

in Nigeria, respectively. There are about 200 species of *Vernonia*. *Vernonia amygdalina* is one of them and a shrub or small tree of 2–5 m with petiolate leaf of about 6 mm diameter and elliptic shape. The leaves are green with a characteristic odour and a bitter taste. No seeds are produced and the tree has therefore to be distributed through cutting (Bonsi *et al.*, 1995). Grows under a range of ecological zones in Africa and produces large mass of forage and is drought tolerant (Bonsi *et al.*, 1995; Akinpelu, 1999).

2.10.8.1 Uses of *Vernonia amygdalina*

The leaves are used for human consumption and washed before eating to get rid of the bitter taste. They are used as vegetable and stimulate the digestive system, as well as they reduce fever. Furthermore, they are used as local medicine against leech, which are transmitting bilharziose (Akinpelu, 1999). Free living chimpanzees eat the leaves, if they have attacked by parasites. *Vernonia amygdalina* is also used, instead of hops to make beer in Nigeria (Ologunde *et al.*, 1992). Furthermore, *Vernonina amygdalina* is found in homes in villages as fence post and pot-herb (Ologunde *et al.*, 1992).

However, in general has been found, that *Vernonia amygdalina* have an astringent taste, which affects its intake (Bonsi *et al.*, 1995). The bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides (Buttler and Bailey, 1973; Bonsi *et al.* 1995). It has been tried to mix *Vernonia* with molasses to make it more palatable, but 6.6 % of DM intake had to be added to improve the intake of *Vernonia* (Buttler and Bailey, 1973; Bonsi *et al.*, 1995).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Laboratory

Phytochemical activities and bioassays were conducted in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, while mosquito larval rearing was carried out in the Department of Biological Sciences, Ahmadu Bello University, Zaria.

3.2 Plant Collection and Identification

Based on the ethnomedical survey reports of plants with insecticidal properties (Dalziel, 1937; Abubakar, 1997; Saidu, 2002; Wiesman and Chapagain, 2003; Shaalan *et al.*, 2005), selected number of plant materials were obtained from Zango, Hanwa, Samaru, Bomo, Danmagaji and Dakache areas of Zaria, Kaduna State. Plants materials were identified in the Herbarium Unit of the Department of Biological Sciences, ABU, Zaria as: *Annona senegalensis* Pers., *Hibiscus lunariifolius* Willd, *Hibiscus asper* Hook.f., *Vernonia amygdalina*, *Hibiscus cannabinus* Linn, *Hibiscus sabdariffa* Linn, *Urena lobata* Linn, *H. esculentus* Linn (Table 3.1). Appropriate specimens of the samples were deposited at the Herbarium and assigned voucher numbers as indicated in Table 3.1.

Table 3.1: Experimental Plants and their Reported Uses

SN	Botanical name	Common name	Local name	Family	Part used	Reported usage	Reference	Voucher no.
1	<i>Annona senegalensis</i> Pers	Custard apple	Gwandar daji	Annonaceae	Leaf	Insect antifeedant	Klaus and Adala, 1994	1250
2	<i>Hibiscus asper</i> Hook	Bush sorrel	Yakuwa	Malvaceae	Leaf	Insecticidal	Foyet <i>et al.</i> , 2011	2240
3	<i>Hibiscus cannabinus</i> Linn	Kenaf	Rama	Malvaceae	Leaf	Insecticidal/Antidiabetic	Mayset <i>et al.</i> , 1990	7942
4	<i>Hibiscus esculentus</i> Linn	Okra	Kubewa	Malvaceae	Leaf	Antideuretic/Insecticidal	Orwa <i>et al.</i> , 2009	12051
5	<i>Hibiscus lunariifolius</i> Willd	Hemp-leaved Hibiscus	Kasheshe	Malvaceae	Leaf	Insecticidal	Exell and Meeuse, 1961	1804
6	<i>Hibiscus sabdariffa</i> Linn.	Sorrel	Sobarodo	Malvaceae	Leaf	Antihypertensive/Insecticidal	Salah <i>et al.</i> , 2002	976
7	<i>Urena lobata</i> Linn	Caesar weed	Karelawar maciji	Malvaceae	Leaf	Insect repellent	Onoagbe <i>et al.</i> , 2010	678
8	<i>Vernonia amygdalina</i> Del	Bitter leaf	Shuwaka	Asteraceae	Leaf	Antidiabetic/Insecticidal	Ologunde <i>et al.</i> , 1992	618

3.3 Collection and Identification of Adult Mosquitoes

Gravid females of *Culex* were collected early in the morning between 6.00am to 8.00am using aspirator from the classrooms of the Faculty of Science and the Faculty of Education, Ahmadu Bello University, Zaria. They were introduced into Rearing Cages containing water in one litre plastic containers for egg laying in the Entomology and Parasitology of Biological Sciences, ABU, Zaria. After egg laying, the hatched larvae were fed with pulverized (powdered) fish feed until they developed into third instar larvae. The third larvae instar of *Culex quinquefasciatus* were identified based on the following features:

- i. Head: upper and lower hairs have 5 or more branches;
- ii. Comb scales: blunt; many in a patch;
- iii. Anal segment: lateral hair single;
- iv. Siphon: index 3.8-4.0; bulbous; 4 pairs of hair tufts; 2 basal tufts multi-branched;
- v. Gills: blunt; longer than anal segment (Hopkins, 1952).

3.4 Rearing of Mosquitoes

Gravid female mosquitoes were introduced into the rearing cage (Plate I) for egg laying. In the cage, water was provided in a one litre plastic container for egg laying. After hatching, the larvae were kept in plastic buckets half filled with tap water and fed with Baker's Yeast once in a day initially, and twice during the later stages of development (WHO, 2005). Larvae were allowed to develop into pupae and later emerged into adults. One-sided shaved quail birds were tied for 6 hrs to the surface of net containing adult mosquito and they fed on the blood of quail birds.

3.5 Preparation of Plant Extracts

Ethanolic extraction was carried out in order to obtain crude extracts of the plants. The leaves of each plant were air-dried for three days and ground into powder. Five hundred grammes(500g) of each powdered plant material were placed in an extraction flask and three litres (3L)of ethanol (95%) was added. The mixture was allowed to stand for 24 hr,with occasional shaking(Brain and Turner, 1975). The extract was filtered and marc pressed. The solvent was evaporated to dryness under reduced pressure at 40°C on a rotatory evaporator. In addition, the aqueous extract of *Annona senegalensis* leaf was prepared and its activity compared with that of the ethanolic extract.

3.6 Preliminary Phytochemical Screening of the Plant Extracts (Ciulei, 1991)

Phytochemical screening was carried out on crude extracts of all the eight selected plants to determine the types and proportion of chemical constituents present:

3.6.1 Test for Alkaloids

Three (3) drops of Mayer's reagent were added to 0.5ml of test solution in a test tube. A yellowish-white precipitate was indication of the presence of alkaloids (Plate II).

3.6.2 Test for Flavonoids

Three (3) strips of metal magnesium were added to 2ml of test solution followed by 6 drops of concentrated HCl acid. A red colouration indicated the presence of flavonoids.

3.6.3 Test for Saponins

Two millilitres (2ml) of the aqueous solution was diluted and shaken in a test tube (1.6cm diameter) for 15 min. A foam column of 1 cm in height that persists for 15 min, indicated the presence of saponins.

3.6.4 Test for Steroids and Triterpenes

Two (2ml) of concentrated H₂SO₄ acid was added to 5ml of the chloroform solution of the extract. A brown ring formed indicated the presence of steroids and triterpenes.

3.6.5 Test for Tannins

Three drops of Ferric chloride were added to 1ml of test solution. A blue colouration indicated the presence of tannins.

3.7 Chromatography (Mahranet *al.*, 1980)

A thin layer of absorbent was prepared by spreading slurries of silica-gel to glass plates (5 x 20cm). The plates were air-dried for 20 min and then activated by heating in an oven to 110°C for 12hr. One hundred (100mg) of crude extract was hydrolyzed in 200ml of 2N Sulphuric acid, by heating over a heating table for two hrs. Using capillary tubes, samples of crude extract were applied at the posterior ends of duplicate chromatoplates. The plates were placed in a developing chamber containing the solvent system; n-Hexane: Ethyl acetate (60:40: v/v). The plates were visualized for spots in iodine vapour, and later, under ultraviolet (UV) light, after spraying with vanillin-Sulphuric reagent and Ferric chloride and heating at 105°C for 5 min.

The Retention factor (Rf value) of spots was calculated as follows;

$$\text{Rf value} = \frac{\text{distance moved by sample}}{\text{solvent front}} \text{ (Mahranet } *al.*, 1980)$$

Separation of the chemical constituents was carried out by Column chromatography.

3.8 Larvicidal Effect of Plant Extracts (WHO, 2005)

The larvicidal bioassay followed the World Health Organization (WHO, 2005) standard protocols. Batches of 25 early fourth instar larvae *Culex quinquefasciatus* were initially exposed to a wide range of test concentrations (10mg/ml- 0.001mg/ml) and a control (water) to find out the activity range of the materials under test (Plate III). A narrower range of 4 concentrations (10mg/ml, 5.0mg/ml, 2.5mg/ml and 1.25mg/ml) yielding between 10% and 95% mortality in 24 hr was used to determine LC₅₀ values. Four replicates were set up for each concentration and the control (without taste material). The test containers were held at 25–28°C under a photoperiod of approximately 12 h light followed by 12 h dark (12L:12D). After the 24 hr exposure, larval mortality was recorded.

Moribund larvae were counted and added to dead larvae for calculating percentage mortality. Dead larvae are those that cannot be induced to move when they were probed with a needle in the siphon or the cervical region. Moribund larvae were those incapable of rising to the surface or not showing the characteristic diving reaction when the water was disturbed. The lethal concentrations of the test solutions and that of Dichlorvos (a standard larvicide) were determined.

3.9 Morphological and Histological tests (Fallatah, 2010)

To assess the effect of plant extracts on the morphology and histology of larvae, the larvae treated with ethanol extract of *Annona senegalensis* leaf were fixed, dehydrated and mounted in paraffin blocks. Eight micrometer (8µm) thick longitudinal sections (LS)

of larval tissue blocks were cut, mounted on glass slides and stained with haematoxylin and eosin for histological examination using a Binocular compound microscope (x1000) fitted with a 12Mpx digital camera. This facilitated effective assessment of defects on the head (e.g., head capsule, mouthparts) and the gut (e.g., epithelium, microvilli, vesicles) regions. Consequently, a valid conclusion was drawn on the effect of the extracts.

3.10 Inhibition of Adult Emergence (IE) (WHO, 2005)

Larval mortality or survival was counted every other day until the complete emergence of adults. The test containers were held at 25–28°C under a photoperiod of 12 hr light followed by 12 hr dark (12L:12D). At the end of the observation period, the impact was expressed as the percentage of larvae that did not develop successfully into viable adults (IE%). In computing the IE% for each concentration, moribund and dead larvae and pupae, as well as adult mosquitoes not completely separated from the pupal case, were considered as “affected” (WHO, 2005). The number of successfully emerged adults was counted from the empty pupal cases. The experiment ended when all the larvae or pupae in the controls died or emerged as adults. Any deformities or morphogenetic effects that occur in either the moulting immature mosquitoes or the emerging adults were recorded. The data from all replicates of each concentration were combined. Total emergence inhibition was calculated on the basis of the number of larvae exposed. The IE% was calculated using the following formula (Mulla *et al.*, 1975):

$$IE (\%) = 100 - [T \times 100/C]$$

Where,

T= percentage survival or emergence in treated batches and

C= percentage survival or emergence in the control.

3.11 Effect of Plant Fractions on Oviposition by *Culex quinquefasciatus*

Gravid females of *Culex quinquefasciatus* that emerged from the ethyl acetate fraction (LC₅₀ 3.7 mg/ml) of *Annona senegalensis* leaf and normal normal gravid females were made to oviposit in clear water. The effect of the ethyl acetate fraction on oviposition was determined to assess fecundity. Adult mosquitoes were fed with 10% sugar solution for the first four days after which the females were fed with blood from a restrained *Coturnix coturnix japonicum* (Quail), in accordance with WHO (2005). One sided shaved quail birds were tied for 6 hrs to the surface of net containing adult mosquito and they fed on the blood of quail birds.

3.12 Isolation of Compound

Eluted ethyl acetate fraction of the leaves of *Annona senegalensis* was mixed with activated charcoal, dissolved in methanol and filtered. The filtrate was air dried and on chromatography elution on preparative Thin Layer Chromatography (TLC) led to the isolation of clear crystals that dissolved in chloroform. The R_f value 0.57 Spectral Data on Proton (¹H, 400 MHz) and Carbon (¹³C, 100 MHz) were collected on Agilent-NMR-vnmrs400. The activity of the compound in test solution was measured against the fourth instar larvae of *Culex quinquefasciatus* using distilled water and 0.1% Dichlorvos as negative and positive control, respectively (Brain and Turner, 1975).

3.13 Data Analyses

All the mortalities were counted, recorded and expressed in percentages in lethality studies. Probit analysis was used to determine the median lethal concentration LC₅₀ (Finney, 1971) of the chemical constituents. One way analysis of variance (ANOVA) was

performed to determine the significant difference in the effects of the test concentrations on the larvae of *Culex quinquefasciatus* in larvicidal activity, inhibition of adult emergence and biological activity of ergosterol. The Means were separated using Duncan Multiple Range Test (DMRT) at $p \leq 0.05$. Student T-test was used to determine significant difference between aqueous extracts and ethanol extracts of *Annona senegalensis* leaf. Mean and standard deviation were calculated and plotted in bar charts to show comparison of oviposition differences between normal females of *Culex quinquefasciatus* and those that emerged from ethyl acetate fraction of *Annona senegalensis* leaf.

CHAPTER FOUR

4.0

RESULTS

4.1 Extract Yield of the Ethanolic Crude Extract of Selected Plants

The yield of eight selected plant samples is shown in Table 4.1. *Hibiscus cannabinus* and *V. amygdalina* produced 142.00g each (the highest yield) followed by *A. senegalensis* (134.00g), *H. sabdariifa* (121.00g), *U. lobata* (112.00g), *H. asper* (104.00 g), *H. lunariifolius* (91.00g) and the least yield was recorded in *H. esculentus* (89.00g).

4.2 Extract Yield of Partitioned Crude Ethanolic Extracts of Selected Plants

The yield of the five fractionated plant extracts using different solvents is shown in Table 4.2. There was degree of variation in the yield of the solvents per plant type. Aqueous fraction (31.00g) of *A. senegalensis* had the highest yield followed by N-butanol (13.00g, ethyl acetate (6.20g) and the least was found in hexane (3.00). Aqueous fraction (22.00) of *H. asper* was also higher than ethyl acetate (5.00g), N-butanol (4.30g) and while the lowest was observed in hexane solvent (2.80g). A similar pattern was recorded in the fraction of *H. esculentus* with aqueous having the highest value of 31.00g, while, N-butanol, ethyl acetate and hexane yielded 8.10g, 6.70g and 3.10g respectively. *Urena lobata* fractions yielded 29.00g in aqueous, 11.00g in N-butanol, 5.30, for ethyl acetate and the least was found in hexane with 3.10g. The same trend was recorded in *V. amygdalina* fractions with aqueous fraction having 31.00g as highest, while others were in decreasing order of N-butanol (13.00g) > ethyl acetate (6.20g) > hexane (3.00g).

Table 4.1: Extract yield of the ethanolic crude extracts of the selected plants

S/N	Plant	Extract (g)	% Yield (g)
1	<i>A. senegalensis</i>	134.00	26.8
2	<i>H. asper</i>	104.00	20.8
3	<i>H. cannabinus</i>	142.00	28.4
4	<i>H. esculentus</i>	89.00	17.8
5	<i>H. lunariifolius</i>	91.00	18.2
6	<i>H. sabdariffa</i>	121.00	24.2
7	<i>U. lobata</i>	112.00	22.4
8	<i>V. amygdalina</i>	142.00	28.4

N.B: Quantity of powder 500g; volume solvent = 2 litres

Table 4.2: Extract yield of partitioned ethanolic crude extracts of selected plants

Plants	Solvents	Solvent Quantity (L)	Fraction (g)	% Yield
<i>A. senegalensis</i>	Aqueous	0.5	31.00	38.75
	Ethyl acetate	1.5	6.20	7.75
	Hexane	1.5	3.00	3.75
	N-butanol	2.5	13.00	16.25
<i>H. asper</i>	Aqueous	0.5	22.00	27.50
	Ethyl acetate	1.5	5.00	6.25
	Hexane	1.5	2.80	3.50
	N-butanol	2.0	4.30	5.38
<i>H. esculentus</i>	Aqueous	0.6	31.00	38.75
	Ethyl acetate	1.5	6.70	8.38
	Hexane	1.5	3.10	3.88
	N-butanol	2.5	8.10	10.13
<i>U. lobata</i>	Aqueous	0.5	29.00	36.25
	Ethyl acetate	1.5	5.30	6.63
	Hexane	1.5	2.90	3.63
	N-butanol	2.5	11.00	13.75
<i>V. amygdalina</i>	Aqueous	0.5	31.00	38.75
	Ethyl acetate	1.5	6.20	7.75
	Hexane	1.5	3.00	3.75
	N-butanol	2.5	13.00	16.25

N.B: Quantity of powder = 80 g

4.3 Phytochemical Constituents of Selected Plant Extracts

The qualitative constituents of the selected plant extracts used in this study are shown in Table 4.3. Carbohydrates were present (+) in all the plants sampled. Anthracenes were present in low quantity (+) in *Vernonia amygdalina* and *Annona senegalensis* only. Combined Anthracenes was present (+) in small quantity in *V. amygdalina* only. The Unsaturated triterpenes were found in different proportions in all the plant extracts. They were present in high quantity (++) in *H. cannabinus*, *H. esculentus*, *H. sabdariffa*, *Urena lobata*, *V. amygdalina*, and *A. senegalensis* but low (+) in *H. asper* and *H. lunariifolius*. Unsaturated sterols were found in high quantity (+) in plants except in *H. asper*, *H. cannabinus* and *H. lunariifolius* where the quantity was low (+). The amount of cardiac glycosides was low (+) in *H. asper*, *H. lunariifolius*, *H. sabdariffa* and *V. amygdalina* but high (++) in *H. cannabinus*, *H. esculentus*, *U. lobata* and *A. senegalensis*. Saponins were present in different proportions in all the plants. They were in high quantity (++) in *U. lobata* and *A. senegalensis* and low quantity (+) in *H. asper*, *H. lunariifolius*, *H. sabdariffa*, *H. esculentus*, *H. cannabinus* and *V. amygdalina*. Tannins were present in low quantity (+) in *H. asper*, *H. esculentus*, *H. lunariifolius* and *H. sabdariffa* but high quantity (++) in *H. cannabinus*, *U. lobata*, *V. amygdalina* and *A. Senegalensis*. Flavanoids were present in low quantity (+) in *H. asper*, *H. cannabinus*, *H. esculentus*, *H. lunariifolius*, and *H. sabdariffa*, while in *A. senegalensis* they were in high quantity (++)). Alkaloids were present in low quantity (+) in *Hibiscus cannabinus*, *H. sabdariffa* and *A. senegalensis*.

Table 4.3: Qualitative Phytochemical Profile of Selected Plants Species

Phyto	H. asper	H. can.	H. esc.	H. lun.	H. sabd.	U. lob	V. amyg	A. seneg
Carbohyd.	+	+	+	+	+	+	+	+
Anthra	-	-	-	-	-	-	+	+
Com Anth	-	-	-	-	-	-	+	-
USAT	+	++	++	+	++	++	++	++
Un Sterols	+	+	++	+	++	++	++	++
Card Glyc	+	++	++	+	+	++	+	++
Saponins	+	+	+	+	+	++	+	++
Tannins	+	++	+	+	+	++	++	++
Flavonoids	+	+	+	+	+	-	-	++
Alkaloids	-	+	-	-	+	-	-	+

Key:

+ = Present in low quantity, ++= Present in high quantity, - = Absent

Carbohyd= Carbohydrates, Com Anth= Combined Anthracenes, USAT=Unsaturated Triterpenes, Un Sterols= Unsaturated Sterols, Card Glyc= Cardiac Glycosides, H. asper = *Hibiscus asper*, H. can =*Hibiscus cannabinus*, H. esc =*Hibiscus esculentus*, H. lun = *Hibiscus lunariifolius*, H. sabd = *Hibiscus sabdariffa*, U. lob = *Urena lobata*, V. amyg = *Vernonia amygdalina*, A. seneg = *Annona senegalensis*

4.4 Larvicidal Activity of Selected Plant Extracts Against *Culex quinquefasciatus* Larvae

The larvicidal activity of the plants extracts tested against *Culex quinquefasciatus* larvae is shown in Table 4.4. All the plant extracts tested exhibited a dose-dependent larvicidal activity which was significant ($p < 0.05$) only in *H. asper*, *H. esculentus*, *H. sabdariffa*, *U. lobata* and *V. amygdalina*. Such differences were well marked between the lowest concentration (1.25 mg/ml) and higher concentrations of 5.00 mg/ml and 10.00 mg/ml. The dose-dependent larvicidal action of the extracts of *A. senegalensis*, *H. cannabinus* and *H. lunariifolius* were not significantly different ($p > 0.05$).

4.5 Median Lethal Concentration of Ethanolic Extracts of Selected Plants

The median and upper lethal (LC_{50} and LC_{90}) concentrations of the plant extracts are shown in Table 4.5. The LC_{50} and LC_{90} of *A. senegalensis* was found to be 0.46mg/ml and 5.20mg/ml, accordingly; with regression equation of $Y = 1.212x + 5.412$ and R-value of 0.984. The LC_{50} (4.03mg/ml) and LC_{90} (23.15mg/ml) of *H. asper* was the highest with regression equation $Y = 1.810x + 3.904$ and R-value of 0.951. *Hibiscus cannabinus* LC_{50} and LC_{90} were calculated to be 1.24mg/ml and 11.89mg/ml respectively with the regression equation $Y = 1.302x + 4.880$ as well as R-value of 0.956. That of *H. esculentus* was found to be similar to the one obtained in *A. senegalensis* but it was slightly higher having LC_{50} of 0.57mg/ml and LC_{90} of 8.00mg/ml and the regression equation of $Y = 1.116x + 5.272$, R-value of 0.984.

Table 4.4: Larvicidal Activity of Ethanol Extracts of Selected Plants Against *Culex quinquefasciatus*

Extract	Larval mortality at Concentration (mg/ml)				Mean
	1.25	2.50	5.00	10.00	
<i>A. senegalensis</i>	18.00 ^a ±4.36	19.75 ^a ±3.03	22.50 ^a ±1.44	23.75 ^a ±1.25	21.00±1.39
<i>H. asper</i>	5.50 ^c ±2.40	7.00 ^{bc} ±3.16	13.75 ^{ab} ±2.93	19.75 ^a ±1.25	11.50±1.86
<i>H. cannabinus</i>	11.75 ^a ±4.25	17.00 ^a ±4.08	18.25 ^a ±3.90	22.00 ^a ±1.73	17.25±1.88
<i>H. esculentus</i>	16.00 ^b ±1.96	19.00 ^{ab} ±2.08	21.75 ^a ±1.65	22.75 ^a ±1.11	19.88±1.03
<i>H. lunariifolius</i>	8.75 ^a ±3.73	12.50 ^a ±3.78	17.75 ^a ±3.35	16.00 ^a ±2.12	13.75±1.73
<i>H. sabdariiffa</i>	6.75 ^c ±2.50	13.75 ^b ±2.78	21.00 ^a ±1.22	22.75 ^a ±0.75	16.06±1.87
<i>U. lobata</i>	17.50 ^c ±1.66	19.00 ^{bc} ±0.41	22.00 ^{ab} ±1.16	24.00 ^a ±0.71	20.63±0.82
<i>V. amygdalina</i>	13.00 ^b ±0.41	20.25 ^a ±1.44	24.75 ^a ±0.25	19.75 ^a ±3.01	19.44a±1.32
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Means with different superscripts letters per row are significantly different (p<0.05).

Table 4.5: Median Lethal Concentration of Ethanolic Leaf Extracts of Selected Plants

Extract	LC ₅₀ (mg/ml)	LC ₉₀ (mg/ml)	Regression equation	R-value
<i>A. senegalensis</i>	0.457	5.202	$Y = 1.212x + 5.412$	0.984
<i>H. asper</i>	4.032	23.155	$Y = 1.810x + 3.904$	0.951
<i>H. cannabinus</i>	1.237	11.893	$Y = 1.302x + 4.880$	0.956
<i>H. esculentus</i>	0.571	8.002	$Y = 1.116x + 5.272$	0.984
<i>H. lunariifolius</i>	2.568	61.097	$Y = 0.930x + 4.619$	0.758
<i>H. sabdariffa</i>	2.193	8.226	$Y = 2.229x + 4.240$	0.975
<i>U. lobata</i>	0.625	5.281	$Y = 1.381x + 5.282$	0.958
<i>V. amygdalina</i>	0.536	5.769	$Y = 1.239x + 5.337$	0.255

There was wider variation between LC₅₀ and LC₉₀ of *H. lunariifolius* extracts with 2.57mg/ml and 61.10mg/ml respectively and regression equation of $Y = 0.930x + 4.619$, R-value of 0.758. The LC₅₀ and LC₉₀ of *H. sabdariffa* were obtained as 2.19mg/ml and 8.23mg/ml, respectively; regression equation of $Y = 2.229x + 4.240$ and R-value of 0.975. *Urena lobata* LC₅₀ and LC₉₀ were 0.63mg/ml and 5.28mg/ml correspondingly; $Y = 1.381x + 5.282$ and R-value of 0.958. The LC₅₀ was found to be 0.54mg/ml, while LC₉₀ as 5.77mg/ml with regression equation of $Y = 1.239x + 5.337$ and R-value of 0.255. *Annona senegalensis* was the best with the least both LC₅₀ and LC₉₀ and it was followed by *V. amygdalina* while the highest was found to be in *H. asper*.

4.6 Larvicidal Activities of Ethanolic and Aqueous Extracts of *A. senegalensis* on *Culex quinquefasciatus*

The larvicidal activities of the ethanolic and aqueous extracts of *A. senegalensis* on *Culex quinquefasciatus* is shown in Table 4.6. There was significant difference ($p < 0.05$) in larval mortality tested in the concentrations of ethanolic and aqueous extracts on *A. senegalensis*. Both extracts did not exhibit any significant larval mortality at dose 1.25 mg/ml and 2.50 mg/ml. However, at dose 5.00 mg/ml and 10.00 mg/ml, the ethanolic extract exhibited a significant larvicidal activity than the aqueous extract ($p < 0.05$).

4.7 Effect of *Annona senegalensis* Leaf Extract on the Histology of *Culex quinquefasciatus* Larvae

The cellular structure of normal 4th instar larvae of *Culex quinquefasciatus* are shown in Plates I-IV and that of treated larvae on Plates V-VII. The structure of *Culex quinquefasciatus* cephalic region showed intact head region with compound eye, head capsule and brain compared to the sloughed head capsule of the larva treated with *Annona senegalensis* extracts.

Table 4.6: Larvicidal Activity of Ethanolic and Aqueous Leaf Extracts of *A. senegalensis* on *Culex quinquefasciatus* Larvae

Extracts	Mortality			
	Concentration (mg/ml)	Ethanolic Extracts	Aqueous Extracts	P-value
1.25		1.50	1.50	1.00
2.50		5.50	3.50	0.27
5.00		13.50	5.75	0.02
10.00		24.25	7.00	0.00
Mean		11.18	4.44	0.12

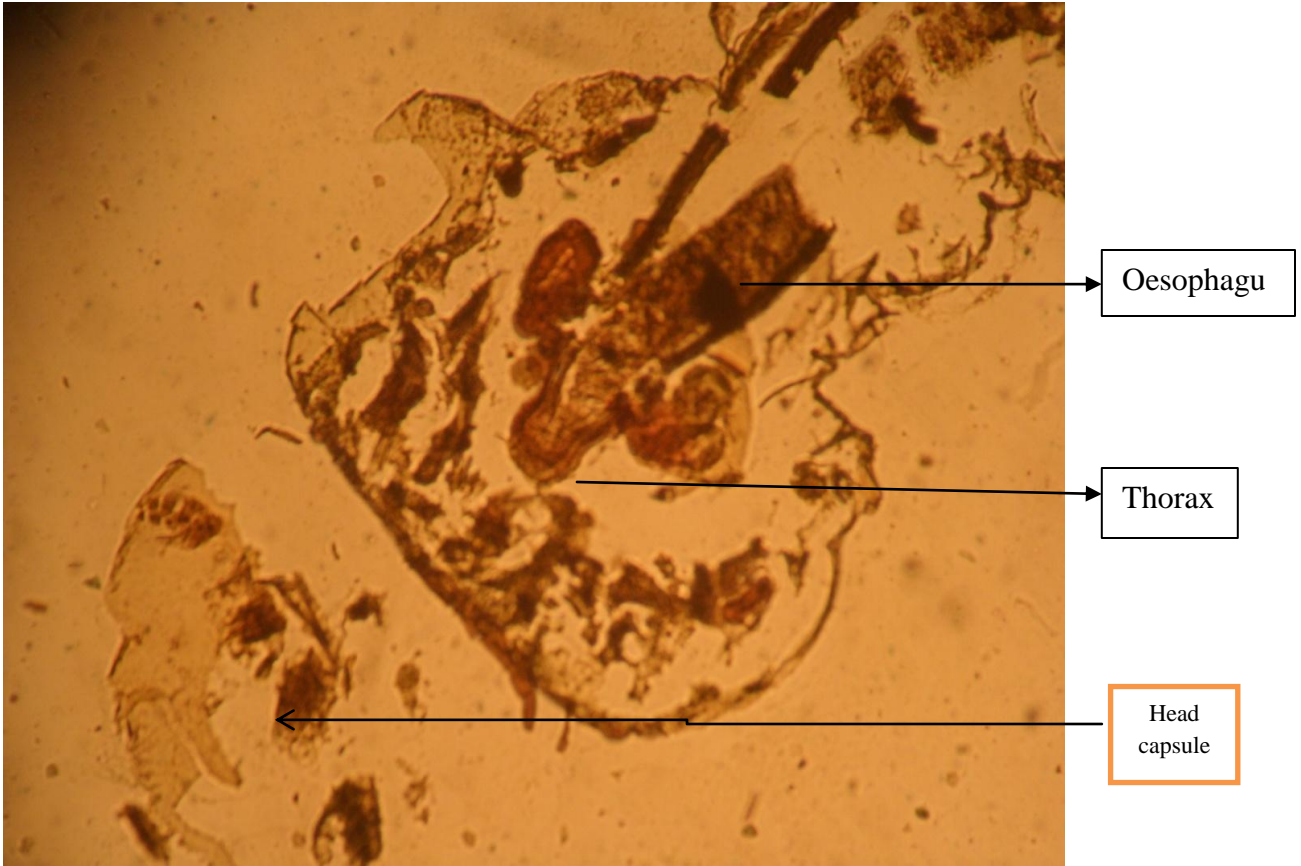


Plate I: Longitudinal Section (L.S) of the cephalic region of normal (control, distilled water) 4th instar larva of *Culex quinquefasciatus*.

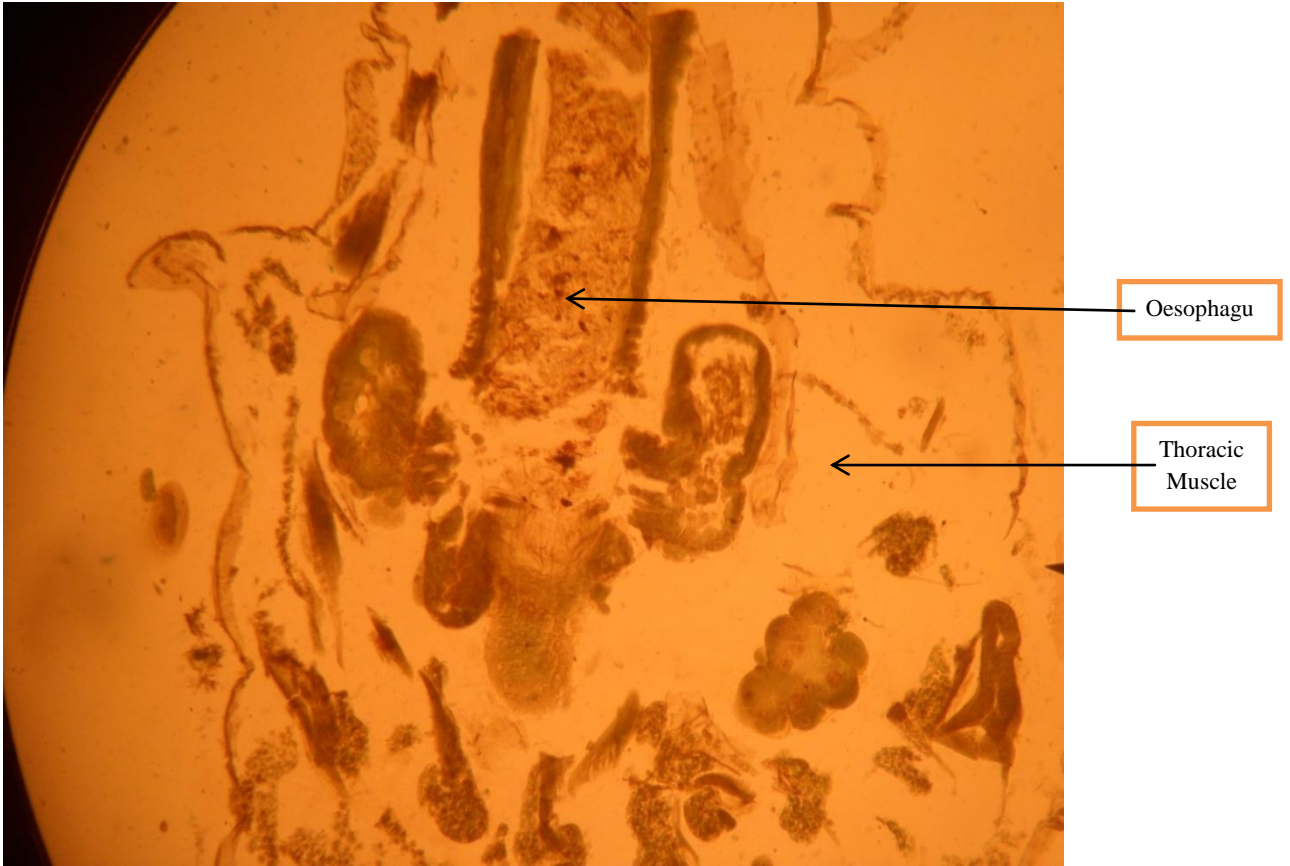


Plate II: Longitudinal Section (L.S) of the thoracic region of normal (control, distilled water) 4th instar larva of *Culex quinquefasciatus*(intact thoracic muscle and oesophagus)

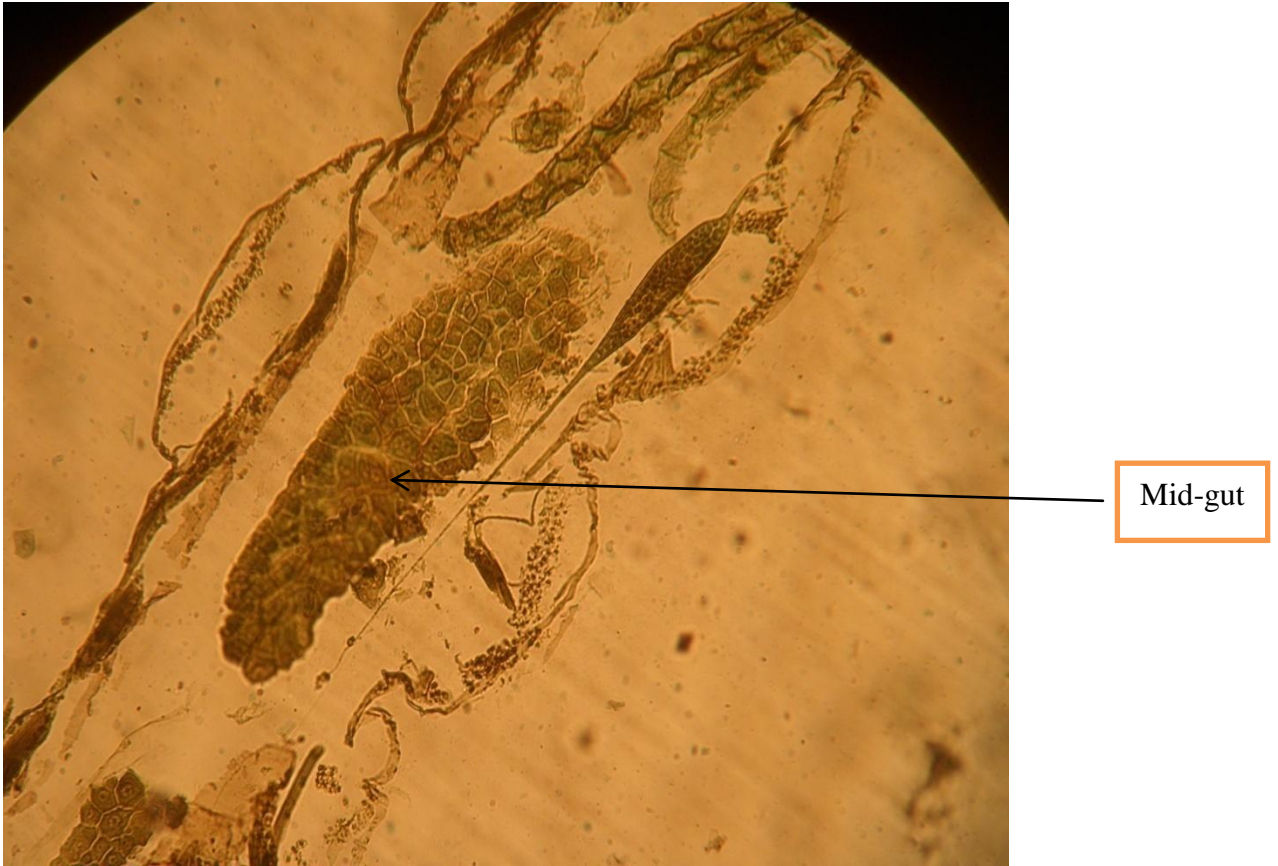


Plate III: Longitudinal Section (L.S) of the mid-gut of the normal (control, distilled water) 4th instar larva of *Culex quinquefasciatus*(intact mid gut).



Plate IV: Longitudinal Section (L.S) of hind gut of normal (control, distilled water) 4th instar larva of *Culex quinquefasciatus*(intact hind gut and anal papillae).

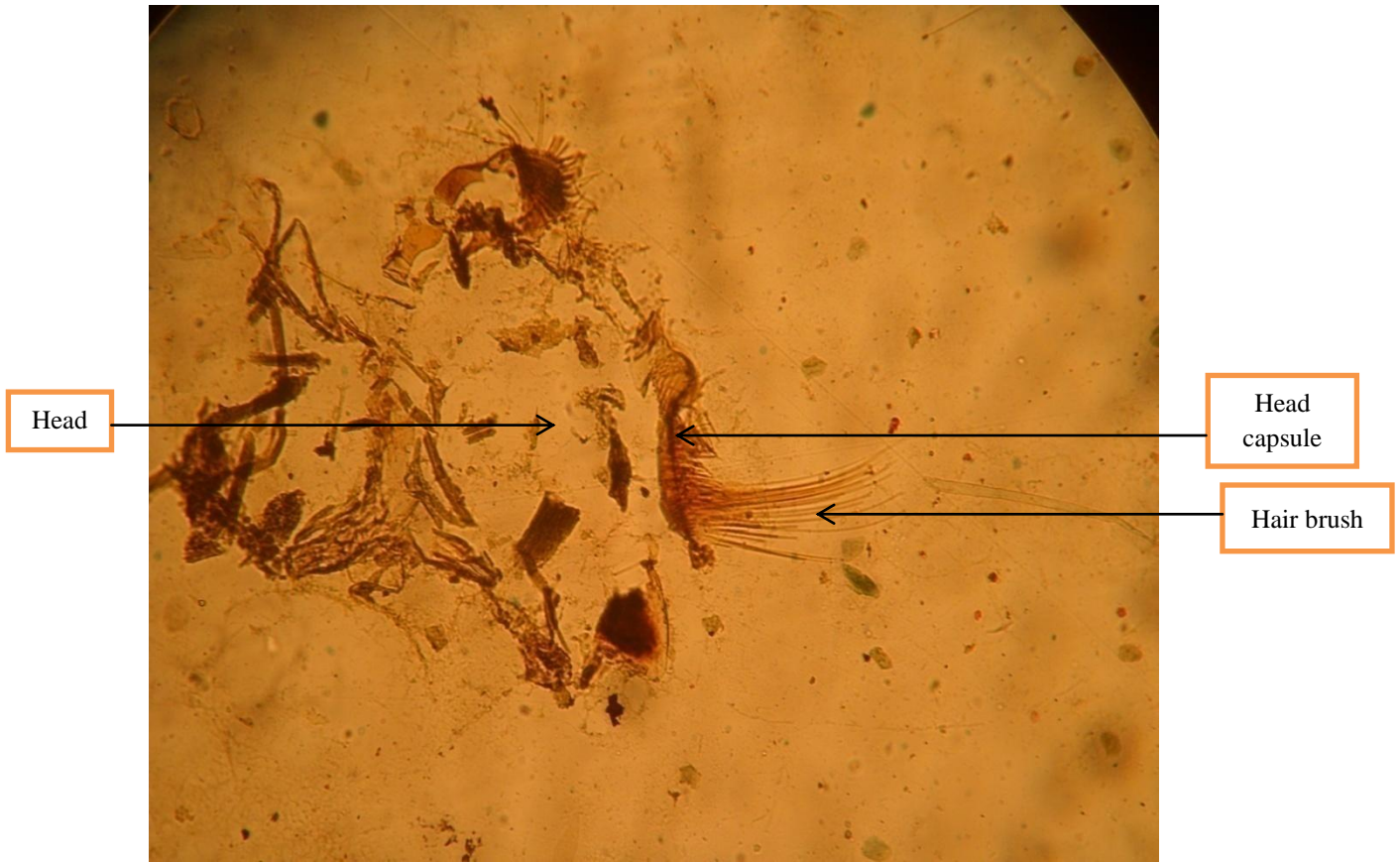


Plate V: Longitudinal Section (L.S) of treated 4th instar larva of *Culex quinquefasciatus* (apolysis of the head capsule) at concentration 10 ml.

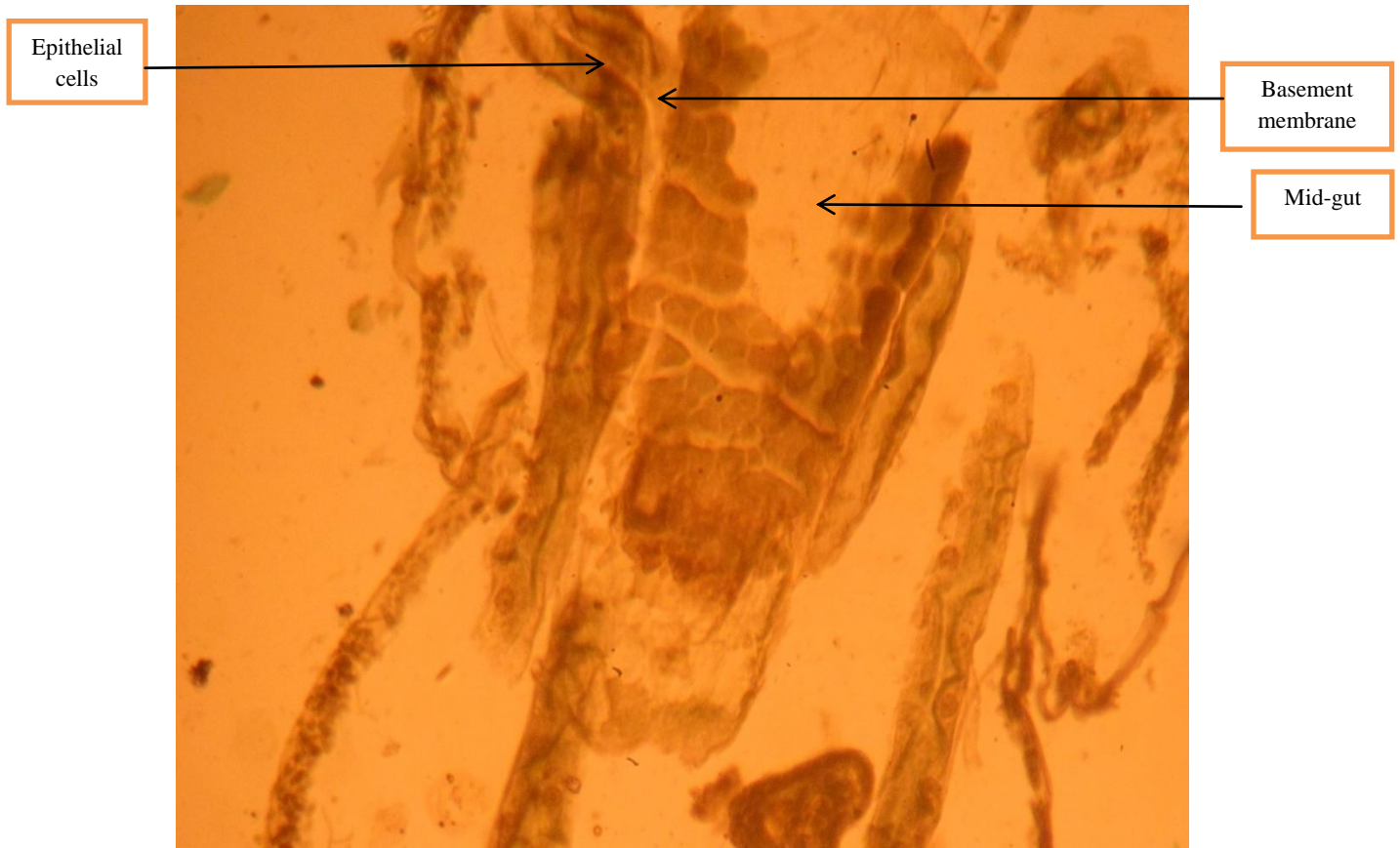


Plate VI: Longitudinal Section (L.S) of Mid region of treated 4th instar larva of *Culex quinquefasciatus*(separation of basement membrane from epithelial cells /degeneration of microvilli) at concentration 10 ml.

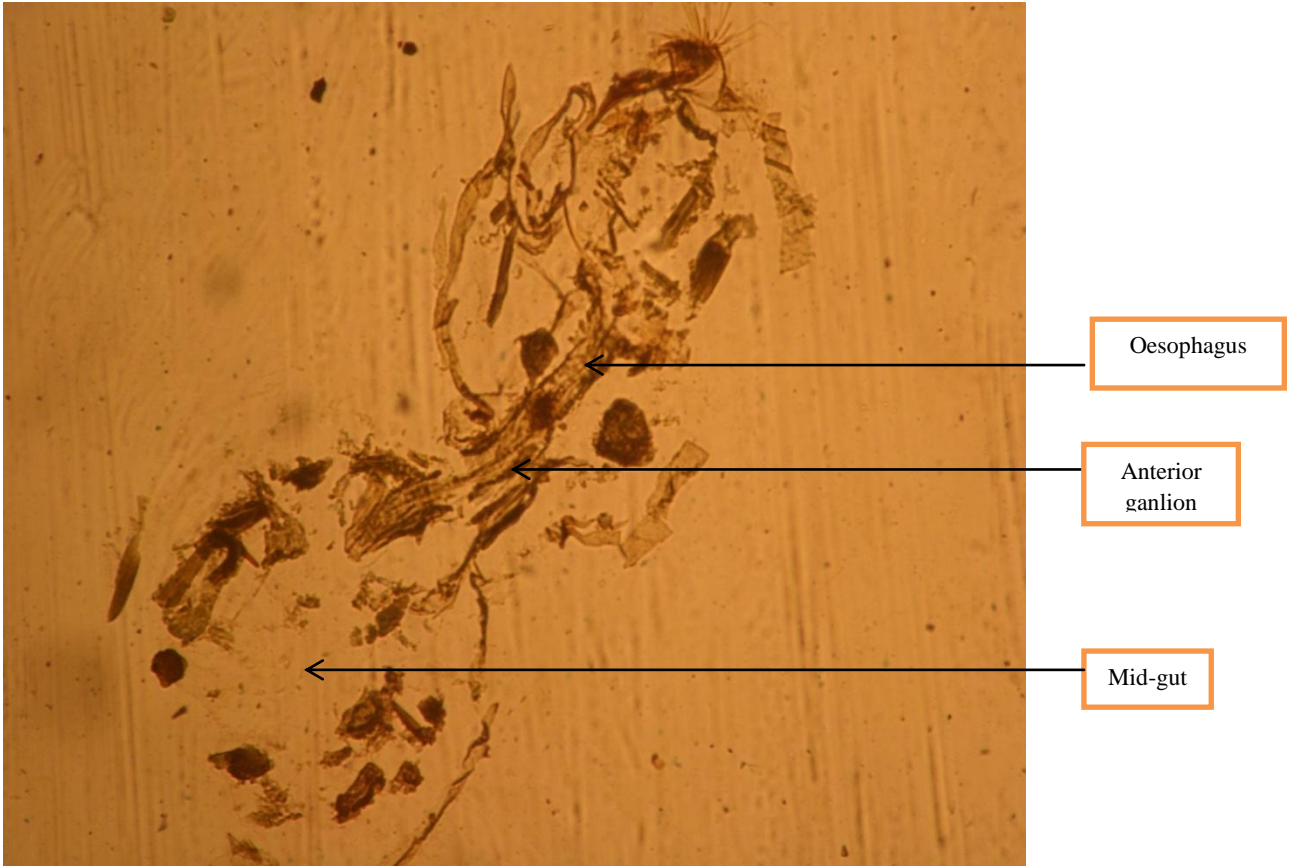


Plate VII: Longitudinal Section (L.S) of treated 4th instar larva of *Culex quinquefasciatus*(advanced degeneration of structures) at concentration 10 ml.

The thoracic region of the control larva presented complete thoracic muscles, mid gut and esophagus, while the treated counterparts showed degenerated muscles, mid gut and esophagus. Abdominal region of untreated larva possessed complete hind gut anal papillae as against total degeneration of hind gut and distorted anal papillae. Other structures that were affected as a result of exposure to *Annona senegalensis* extracts include: damage to anterior ganglion, destruction of epithelial cells, and apolysis of the head capsule.

4.8 Comparison of the Effects of Aqueous, n-Butanol, n- Hexane and Ethyl Acetate Leaf Fractions of *Annona senegalensis* on *Culex quinquefasciatus*

The effects of aqueous, n-butanol, n- hexane and ethyl acetate fractions of *A. senegalensis* on *Culex quinquefasciatus* is shown in Table 4.7. There was significant difference ($p < 0.05$) in mortality of *Culex quinquefasciatus* larvae among the four fractions. At dose 1.25mg/ml, there was significant difference ($p < 0.05$) in n-butanol (3.25) and aqueous fractions (0.00) but were both comparable to n-hexane (1.49) and ethyl acetate (1.50). Ethyl acetate fraction (5.50) and n-hexane (3.15) at 2.50mg/ml were significantly ($p < 0.05$) highest but comparable to n-butanol (2.25) while Aqueous fraction (0.50) was the lowest. There was no significant difference ($p > 0.05$) between Aqueous fraction (0.75) and N-butanol fraction (3.75) at dose 5.00mg/ml, but both were significantly ($p < 0.05$) lower than N-hexane (9.43) and ethyl acetate (13.75). Similar trend was observed at concentration 10.00mg/ml. Aqueous fraction (2.25) and n-butanol fraction did not differ significantly ($p > 0.05$) from each other; similarly n-hexane (13.18) and ethyl acetate (23.50) fractions did not differ significantly ($p > 0.05$) but were significantly ($p < 0.05$) higher than both aqueous (2.25) and n-butanol fractions (5.25).

Table 4.7: Effects of Aqueous, n-Butanol, n- Hexane and Ethyl Acetate Leaf Fractions of *Annona senegalensis* on *Culex quinquefasciatus*

Fraction concentration	Larval mortality				
	Aqueous	n-Butanol	n-Hexane	Ethyl acetate	Average
1.25	0.00 ^b ±0.00	3.25 ^a ±0.95	1.49 ^{ab} ±0.65	1.50 ^{ab} ±0.29	1.58±0.50
2.50	0.50 ^b ±0.29	2.25 ^{ab} ±0.48	3.15 ^a ±0.33	5.50 ^a ±1.19	2.85±0.74
5.00	0.75 ^c ±0.48	3.75 ^c ±0.85	9.43 ^b ±0.38	13.75 ^a ±1.80	6.92±1.79
10.00	2.25 ^c ±1.32	5.25 ^c ±1.32	13.18 ^b ±0.73	23.50 ^a ±0.96	11.05±2.90

Means followed by the same superscript letters per row are not significantly different (p>0.05).

4.9 Inhibition of Adult Emergence in *Culex quinquefasciatus* Exposed to Aqueous Leaf Fraction of *A. senegalensis*

The Inhibition of emergence of *Culex quinquefasciatus* exposed to aqueous fraction of *A. senegalensis* is shown in Figure 4.1. There was variation in inhibitory activity of aqueous fraction of *A. senegalensis* leaf. The number of larvae that was inhibited increased with increase in dose. It was lowest at dose 1.25mg/ml (0.25) followed by 2.50mg/ml (0.42), 5.00mg/ml (0.92) and the highest was dose 10.00mg/ml (2.00). On the contrary, the number of emerged pupae was in reverse order. It was found to be in this order 1.25mg/ml (5.08) > 2.50mg/ml (2.08) > 10.00mg/ml (2.08) > 5.00mg/ml (2.00). Similarly, adult emergence decreased with increase in concentrations. It was highest in concentration 2.50mg/ml (2.08), this was followed by dose 1.25mg/ml (1.83) then at 5.00mg/ml and 10.00mg/ml with adult emergence of 0.25 each.

4.10 Inhibition of Adult Emergence in *Culex quinquefasciatus* Exposed to n-Butanol Leaf Fraction of *A. senegalensis*.

Figure 4.2 is showing the inhibition of *Culex quinquefasciatus* exposed to N-butanol fraction of *A. senegalensis* leaf. The inhibitory activities of n-butanol fraction of *A. senegalensis* differed significantly ($p < 0.05$) among concentrations. Larval mortality increased progressively from 1.25mg/ml to the highest dose (10.00mg/ml). It was in this order of increase: 10.00 mg/ml (5.08) > 5.00 mg/ml (3.83) > 1.25 mg/ml (2.42) > 2.25 mg/ml (2.33).

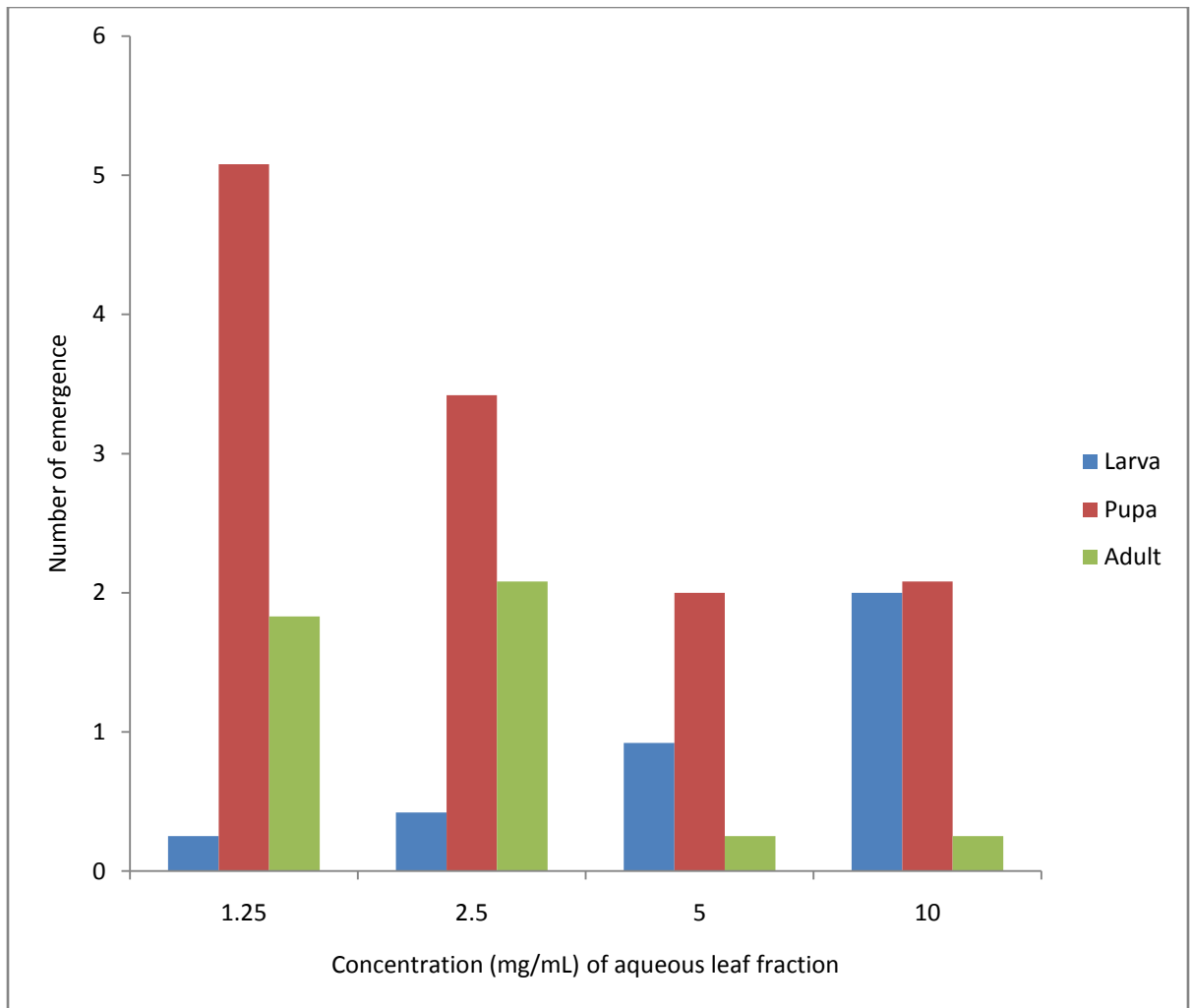


Figure 4.1: Inhibition of adult emergence in *Culex quinquefasciatus* exposed to aqueous leaf fraction of *A. senegalensis*.

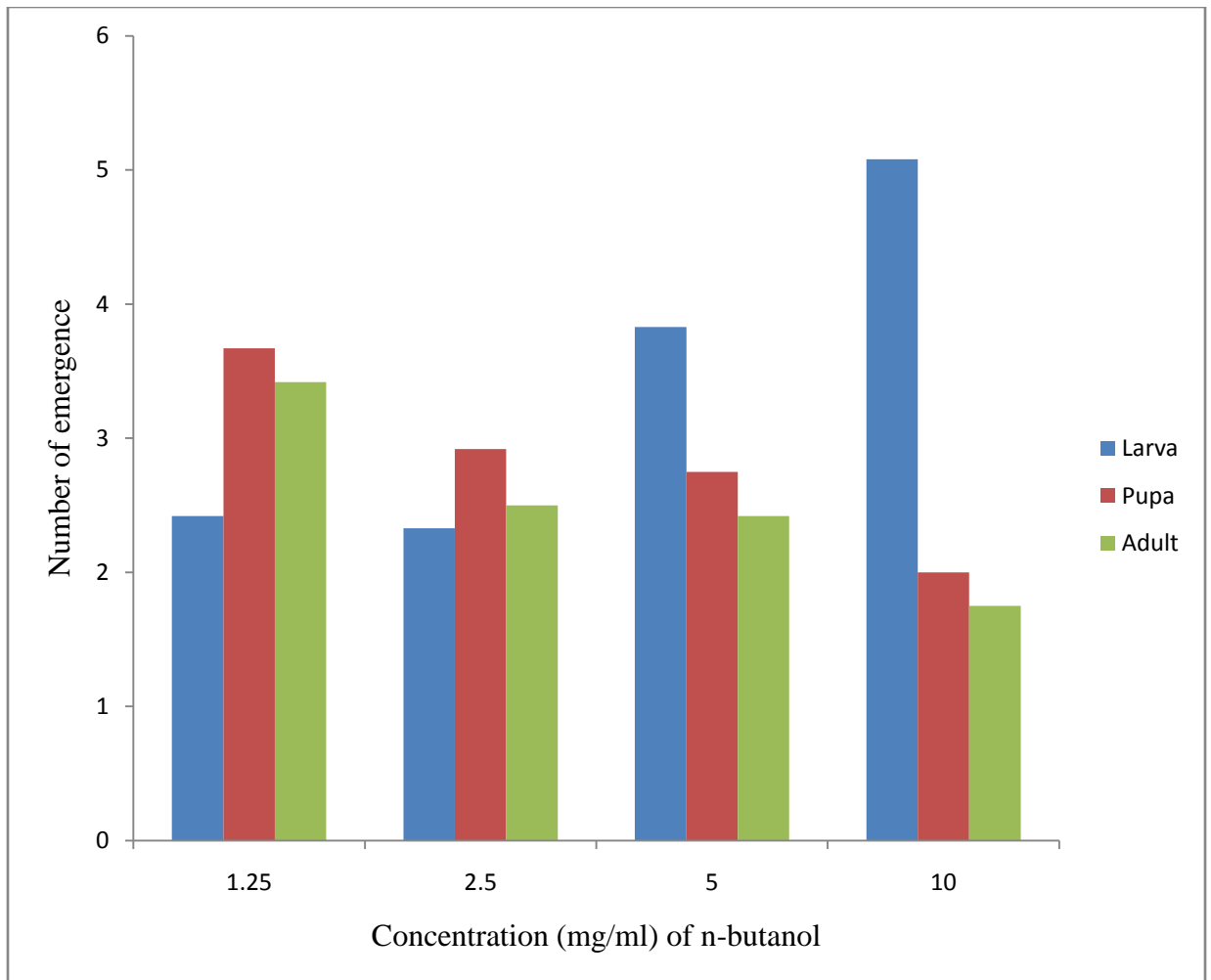


Figure 4.2: Inhibition of adult emergence in *Culex quinquefasciatus* Exposed to n-butanol leaf fraction of *A. senegalensis*

There was decrease in pupal emergence with increase in concentration from 1.25mg/ml to 10.00mg/ml. The number of successful pupal emergence decreased with increase in concentration as follows: 1.25mg/ml (3.67) > 2.50mg/ml (2.920) > 5.00mg/ml (2.75) > 10.00mg/ml (2.00). Adult emergence of *Culex quinquefasciatus* differed significantly ($p < 0.05$) among concentrations of n-butanol fraction of *A. senegalensis*. It was found to be highest at concentration 1.25mg/ml (3.42), it was followed by dose 2.50mg/ml (2.50), 5.00mg/ml (2.42) and 10.00mg/ml (1.75).

4.11 Inhibition of Adult Emergence in *Culex quinquefasciatus* Exposed to n-Hexane Leaf Fraction of *A. senegalensis*

The inhibition of adult emergence by n-hexane fraction of *A. senegalensis* is shown in Figure 4.3. Larval survival was dose dependent. The number of larvae that emerged into pupae decreased with increase in concentration. Lower larval mortality was recorded at dose 1.25 m/mL (7.23) and it increased to 9.08, 13.45 and 15.67 at concentrations 2.5 m/mL, 5.00 m/L and 10.00 m/mL respectively. Pupal survival also observed dose dependent. Higher survival rate was recorded at dose 1.25 m/mL (14.00) it was followed by 7.85 and 5.32 at doses 2.50 m/mL and 5.00 m/mL while least value was obtained at concentration 10.00 m/mL (3.10). The number of successfully emerged adult decreased with concentrations. At dose 1.25 m/mL, 8.57 adult emerged, followed by 2.50 m/mL (3.56), 5.00 m/mL (2.15) and the least were found in 10.00 m/mL (1.08).

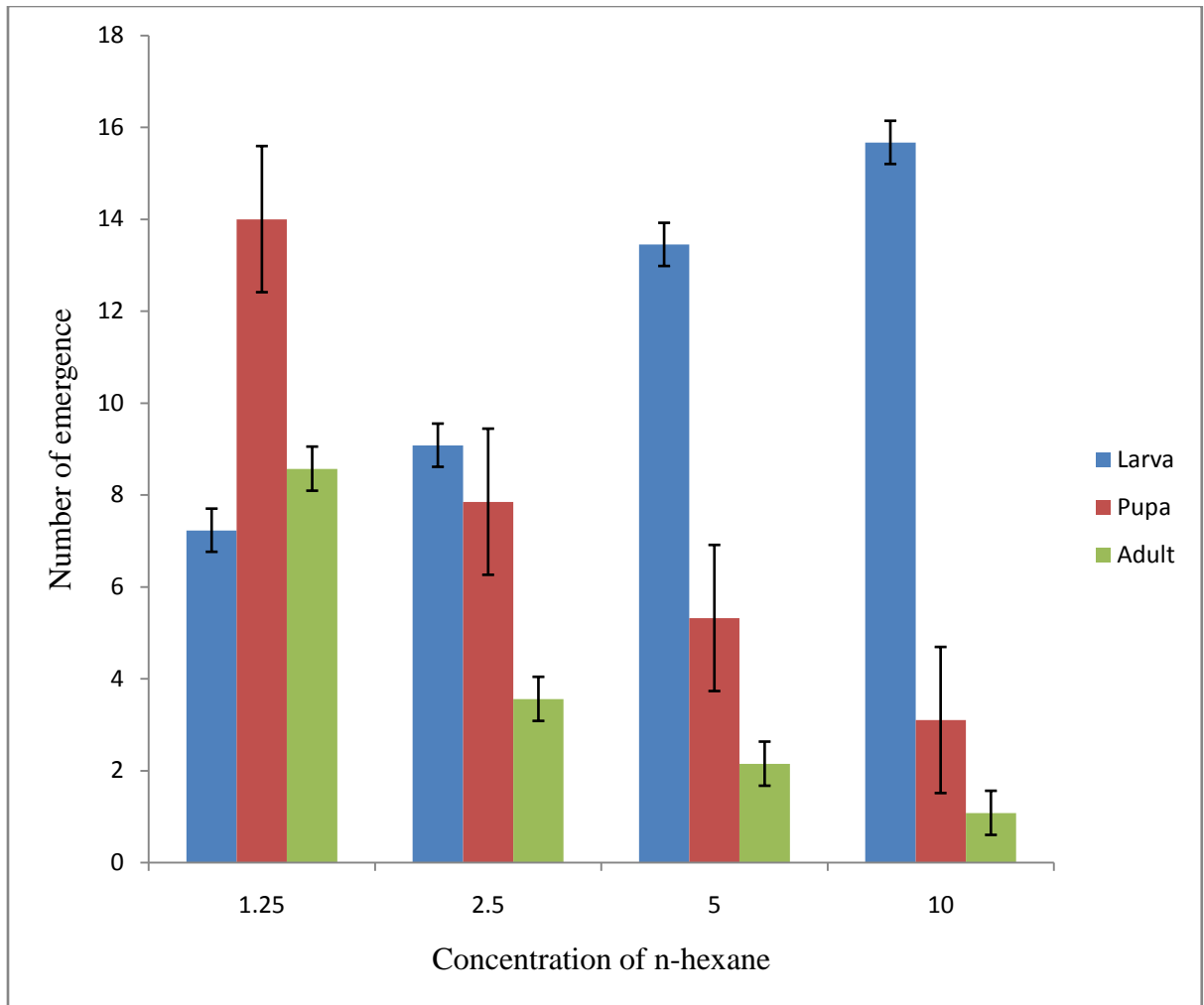


Figure 4.3: Inhibition of adult emergence in *Culex quinquefasciatus* Exposed to n-hexane leaf fraction of *A. senegalensis*

4.12 Inhibition of adult Emergence in *Culex quinquefasciatus* Exposed to Ethyl Acetate Leaf Fraction of *A. senegalensis*

Figure 4.4 shows the inhibition of adult emergence of *Culex quinquefasciatus* exposed to *A. senegalensis* fraction. There was strong inhibitory activity of *A. senegalensis* fractions at all the concentrations on *Culex quinquefasciatus*. Larval mortality was dose-dependent. The number of dead larvae in concentration 1.25mg/ml was found to be 1.58 and was the lowest. Mortality increased to 5.67 at concentration 2.50mg/ml; it further accentuated to 13.58 at concentration 5.00mg/ml and the highest was recorded at 10.00mg/ml with larval mortality of 23.67. There was general decrease in pupal emergence from the lowest concentration to the highest dose. At dose 1.25mg/ml number of successful emerged larvae was 5.58 it was the highest followed by 2.25mg/ml (4.25), 3.42 at 5.00mg/ml and the least was recorded at 10.00mg/ml (0.08). There was further reduction in successfully emerged adult *Culex quinquefasciatus* from pupae. The successfully emerged adults were found to be 2.92, 2.42, 1.50 and 0.08 mg/ml at concentrations 1.25mg/ml, 2.50mg/ml, 5.00mg/ml and 10.00mg/ml, respectively.

4.13 Larvicidal Activity of Ethyl Acetate Leaf Fractions of Selected Plants

The larval mortality of Ethyl acetate fractions of the leaves *A. senegalensis*, *H. asper*, *H. esculentus*, *U. lobata* and *V. amygdalina* against *Culex quinquefasciatus* is shown in Table 4.8. There was significant mortality across all the concentrations of the plant extracts. Larval mortality was dose dependent in *A. senegalensis* fraction. There was significant ($p < 0.05$) mortality of larvae at concentration 10.00 mg/ml.

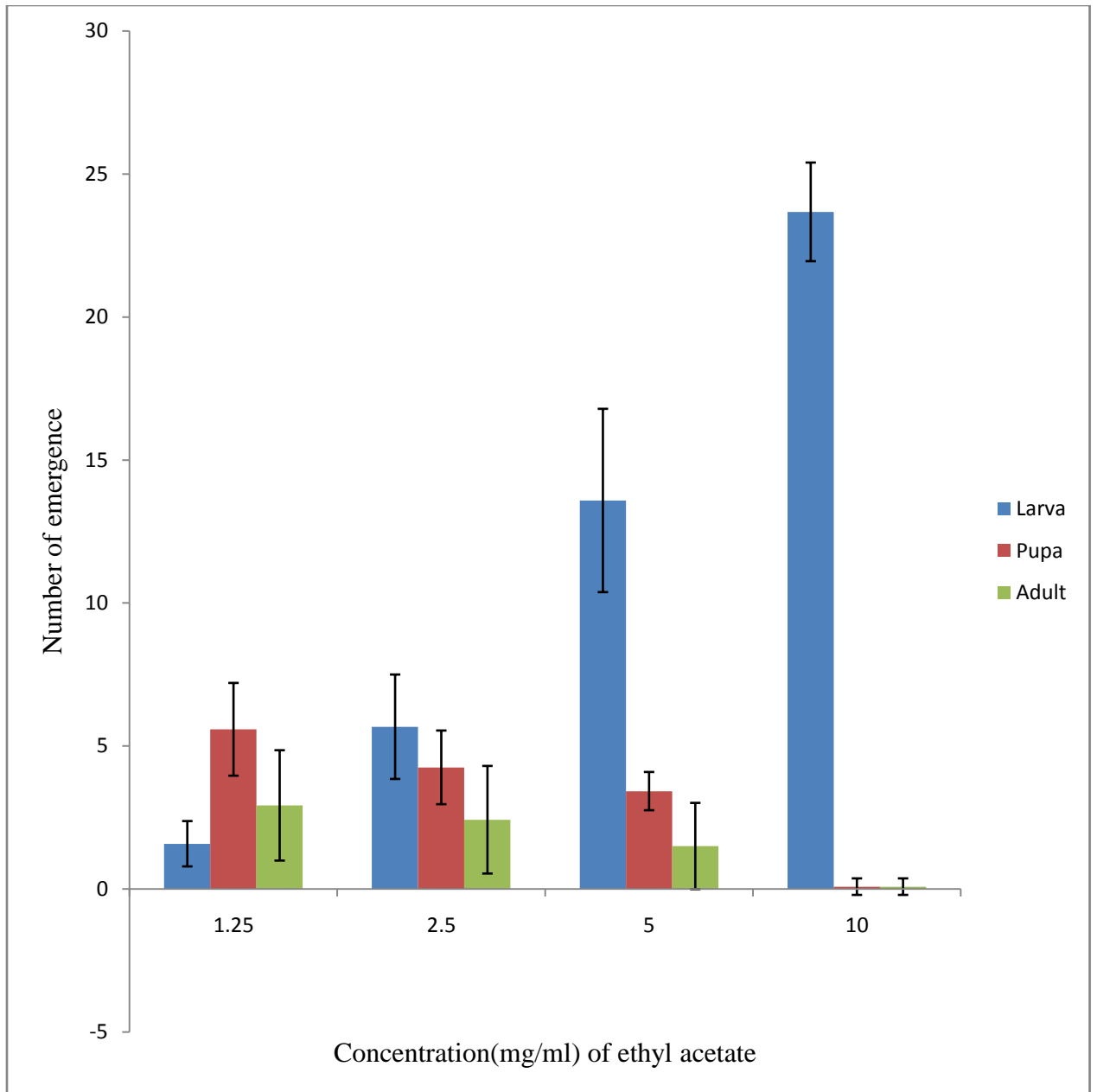


Figure 4.4: Inhibition of adult emergence in *Culex quinquefasciatus* Exposed to ethyl acetate leaf fraction of *A. senegalensis*

Table 4.8: Larvicidal Activity of Ethyl Acetate Leaf Fractions of Selected Plants

Extract	Extract Concentration (mg/ml±SE)			
	1.25 mg/ml	2.50mg/ml	5.00mg/ml	10.00 mg/ml
<i>A. senegalensis</i>	1.50 ^d ±0.29	5.50 ^c ±1.19	13.75 ^b ±1.80	23.50 ^a ±0.96
<i>H. asper</i>	1.75 ^d ±0.25	4.75 ^c ±0.48	7.25 ^b ±0.48	13.75 ^a ±0.48
<i>H. esculentus</i>	1.00 ^c ±0.00	2.00 ^c ±0.58	11.50 ^b ±1.04	21.00 ^a ±0.41
<i>U. lobata</i>	1.25 ^c ±0.25	3.50 ^c ±1.19	12.75 ^b ±1.11	21.75 ^a ±0.63
<i>V. amygdalina</i>	1.25 ^c ±0.25	2.75 ^c ±1.03	12.25 ^b ±1.49	21.50 ^a ±0.65

Means with different superscripts letters per row are significantly different (p<0.05).

This was followed by doses 5.00mg/ml, 2.50mg/ml and 1.25mg/ml with larval mortalities of 13.75, 5.50 and 1.50 respectively. *Hibiscus asper* extracts exhibited the same pattern with *A. senegalensis*. The mortality was significantly ($p<0.05$) highest at dose 10.00mg/ml (13.75) and the lowest was found to be in concentration 1.25mg/ml (1.75). There was significant ($p<0.05$) increase in larval mortality from 1.25mg/ml to 10.00mg/ml in the fraction of *H. esculentus*. Mortality was highest at dose 10.00mg/ml (21.00) and it was significantly ($p<0.05$) higher than 5.00mg/ml. On the contrary, doses 2.50mg/ml (2.00) and 1.25mg/ml (1.00) did not differ significantly ($p<0.05$). Similar trend was observed in *U. lobata*. There was significant ($p<0.05$) difference between doses 10.00mg/ml (21.75) and 5.00mg/ml (12.75), while doses 2.50mg/ml (3.50) and 1.25mg/ml (1.25) did not differ significantly ($p<0.05$). Larval mortality in concentrations of *V. amygdalina* was dose dependent. There was no significant ($p<0.05$) increase in larval mortality between dose 1.25mg/ml (1.25) and 5.00mg/ml (2.75) but they were lower than the one recorded at 5.00mg/ml (12.25) and 21.50 at 10.00mg/ml.

4.14 Effect of Ethyl Acetate Leaf Fractions of Selected Plants on Larval-Pupal Development

The development of larvae to pupae exposed to ethyl acetate fractions of five leaves extracts is shown in Table 4.9. Pupal emergence differed significantly ($p<0.05$) among the plant extracts. The effect of mortality caused by ethyl acetate fraction of *Annona senegalensis* at concentration 1.25 mg/ml (6.25) differ significantly ($p<0.05$) from that caused by 2.50 mg/ml (4.50). There was no significant difference ($p<0.05$) in pupal emergence between concentration 2.50 mg/ml and 5.00 mg/ml (3.50) but differed significantly ($p<0.05$) from dose 10.00 mg/ml with.

Table 4.9: Effect of Ethyl Acetate Leaf Fractions on the Survival of *Culex quinquefasciatus* Larvae to Pupal Emergence

Extract	Extract Concentration (mg/ml±SE)			
	1.25	2.50	5.00	10.00
<i>A. senegalensis</i>	6.25 ^a ±0.48	4.50 ^b ±0.65	3.50 ^b ±0.29	0.00 ^c ±0.00
<i>H. asper</i>	16.25 ^a ±0.63	13.25 ^{ab} ±1.38	12.75 ^b ±0.25	8.00 ^c ±1.08
<i>H. esculentus</i>	9.75 ^a ±0.85	7.25 ^b ±0.48	5.75 ^b ±0.48	1.25 ^c ±0.25
<i>U. lobata</i>	8.00 ^a ±0.71	6.75 ^{ab} ±0.48	5.75 ^b ±0.48	0.50 ^c ±0.29
<i>V. amygdalina</i>	7.25 ^a ±0.63	6.25 ^{ab} ±0.48	5.25 ^b ±0.48	0.25 ^c ±0.25

Means with different superscripts letter per row are significantly different (p<0.05).

n = 25

Pupal emergence in Ethyl acetate fraction of *H. asper* was significantly ($p < 0.05$) highest in concentration 1.25 mg/ml (16.25) but comparable to 2.50 mg/ml (13.25). The emergence at 5.00mg/ml (3.50) was significantly ($p < 0.05$) higher than dose 10.00mg/ml with no emergence. There was significant ($p < 0.05$) pupal emergence at various concentrations of *H. esculentus*. The number of emerged pupae were significantly ($p < 0.05$) highest at 1.25mg/ml. It was followed by 2.50mg/ml (7.25) and 5.00mg/ml (5.75) but they did not differ significantly ($p > 0.05$) from each other, while dose 10.00mg/ml (1.25) recorded the least. All the concentrations of *U. lobata* recorded emergence of pupae. There was no significant ($p < 0.05$) difference in pupal emergence of doses 1.25mg/ml (8.00) and 3.50mg/ml (6.75) in which the latter was in comparable with dose 5.00mg/ml (5.75) and the least was obtained at dose 10.00mg/ml (0.50). The effect of *V. amygdalina* fraction on pupal emergence followed the same trend with the one recorded in *U. lobata* fraction. The emergence was significantly highest at dose 1.25mg/ml (7.25) but in comparison to 2.50mg/ml (6.35). Similarly, dose 5.00mg/ml (5.25) was significantly ($p < 0.05$) higher than 10.00mg/ml (0.25).

4.15 Effect of Ethyl Acetate Leaf Fractions of Plant on the Development of Adult *Culex quinquefasciatus*

Table 4.10 shows the effect of ethyl acetate fractions of some plants' leaves on the development of *Culex quinquefasciatus* pupae to adult. There was no significant ($p > 0.05$) emergence of adult with increase in concentration of *A. senegalensis* but it ranged from 2.25 at 1.25 mg/ml to no adult emergence at dose 10.00 mg/ml.

Table 4.10: Effect of Ethyl Acetate Leaf Fractions of Selected Plants on Adult Emergence in *Culex quinquefasciatus*

Extract	Extract Concentration (mg/ml±SE)			
	1.25mg/ml	2.50 mg/ml	5.00 mg/ml	10.00 mg/ml
<i>A. senegalensis</i>	2.25 ^a ±0.48	2.00 ^a ±1.35	0.75 ^a ±0.48	0.00 ^a ±0.00
<i>H. asper</i>	12.50 ^a ±0.65	10.75 ^a ±0.75	7.75 ^b ±0.48	5.50 ^c ±0.65
<i>H. esculentus</i>	6.00 ^a ±0.71	5.00 ^a ±0.71	3.25 ^b ±0.25	0.75 ^c ±0.25
<i>U. lobata</i>	4.75 ^a ±0.25	4.25 ^a ±0.25	2.75 ^b ±0.25	0.50 ^c ±0.29
<i>V. amygdalina</i>	4.50 ^a ±0.29	4.00 ^a ±0.41	3.50 ^a ±0.29	0.25 ^b ±0.25

Means with different superscripts per row are significantly different ($p < 0.05$).
n = 25

All the concentrations of *H. asper* fractions recorded adult emergence. There was no significant ($p>0.05$) difference in adult emergence between doses 1.25mg/ml (12.50) and 2.50mg/ml (10.75). On the contrary, dose 5.00mg/ml (7.75) and 10.00mg/ml (5.50) differed significantly ($p<0.05$). *Hibiscus esculentus* extracts differed significantly ($p<0.05$) in adult emergence of *Culex quinquefasciatus*. At 1.25mg/ml (6.00) and 2.50mg/ml (5.00) did not differ significantly ($p<0.05$) despite slight reduction in emergence. Dose 5.00mg/ml (3.25) was significantly ($p<0.05$) higher than the emergence recorded at 10.00mg/ml (0.75). *Urena lobata* fraction recorded significant ($p<0.05$) adult emergence from pupae in all the concentrations. There was no significant ($p>0.05$) difference in adult emergence at 1.25mg/ml (4.75) and 2.50mg/ml (4.25) but differed significantly ($p<0.05$) from dose 5.00mg/ml (2.750 while concentration 10.00mg/ml (0.50) recorded the least. *Vernonia amygdalina* fraction exhibited dose dependent adult emergence. There was no significant ($p>0.05$) difference in adult emergence of 1.25mg/ml (4.50), 2.50mg/ml (4.00) and 5.00mg/ml (3.50) but it differed significantly ($p<0.05$) from dose 10.00mg/ml (0.25).

4.16 Effect of Ethyl Acetate Leaf Fraction of *A. senegalensis* on Larvae, Pupae and Adult Emergence of *Culex quinquefasciatus*

The effect of ethyl acetate fraction of *A. senegalensis* on the developmental stages of *Culex quinquefasciatus* larvae is shown in Table 4.11. There was significant difference ($p<0.05$) of mortality in all the concentrations of ethyl acetate fraction of *A. senegalensis* than the negative control (untreated water) but were lower than the positive control (dichlovos).

Table 4.11: Effect of Ethyl Acetate Leaf Fraction of *A. senegalensis* on the Developmental Stages of *Culex quinquefasciatus*

Concentration (mg/ml)	Larval mortality	Pupal survival	Adult emergence	Control (Water)	Control (0.1% Dichlovos)
1.25	1.50 ^d ±0.29	6.25 ^a ±0.48	2.25 ^a ±0.48	25.00±0.00	25.00±0.00
2.50	5.50 ^c ±1.19	4.50 ^b ±0.65	2.00 ^a ±1.35	25.00±0.00	0.00±0.00
5.00	13.75 ^b ±1.80	3.50 ^b ±0.29	0.75 ^a ±0.48	25.00±0.00	0.00±0.00
10.00	23.50 ^a ±0.96	0.00 ^c ±0.00	0.00 ^a ±0.00	25.00±0.00	0.00±0.00
Mean	11.06±2.24	3.56±0.62	1.25±0.41	25.00±9.68	6.25±2.80

Means with different superscripts per column are significantly different (p<0.05).

Larval mortality was significantly ($p < 0.05$) highest at dose 10.00mg/ml (23.50) and it was followed by 5.00 mg/ml (13.75), then 2.50 mg/ml (5.50) while dose 1.25mg/ml (1.50) recorded the least. Pupal emergence differed significantly ($p < 0.05$) among the concentrations. There was significant ($p < 0.05$) emergence of larvae to pupae at dose 1.25mg/ml (6.25) compared to 2.50mg/ml (4.50) and 5.00mg/ml (3.50) in which the latter did not differ significantly ($p < 0.05$) among themselves, while at dose 10.00mg/ml no emergence was observed. Survival of pupae to adult did not differ significantly ($p > 0.05$) within the concentrations. The number of pupae that emerged was highest at 1.25mg/ml (2.25) while 10.00mg/ml did not allow larvae to survive up to adult. All the larvae introduced into control 1 (water) survived to adult stage. In contrast, no larval survival was recorded at control 2 (dichlorvos).

4.17 Inhibition of Adult Emergence in *Culex quinquefasciatus* by Ethyl Acetate Leaf Fractions of Selected Plants

Figure 4.5 is showing the inhibition of adult emergence of *Culex quinquefasciatus* by some ethyl acetate extracts. There was little adult inhibition in control (water), while dichlorvos prevented all adults from emerging. At dose 1.25mg/ml, *H. asper* recorded the least inhibition of adult emergence but it was highest in *A. senegalensis*. Similar trend was obtained at dose 2.50 mg/ml, 5.00 mg/ml and 10.00 mg/ml. The lowest mortality was consistently found in *H. asper* fraction and it increased in this order in other plants: *H. esculentus* < *U. lobata* < *V. amygdalina* < *A. senegalensis*.

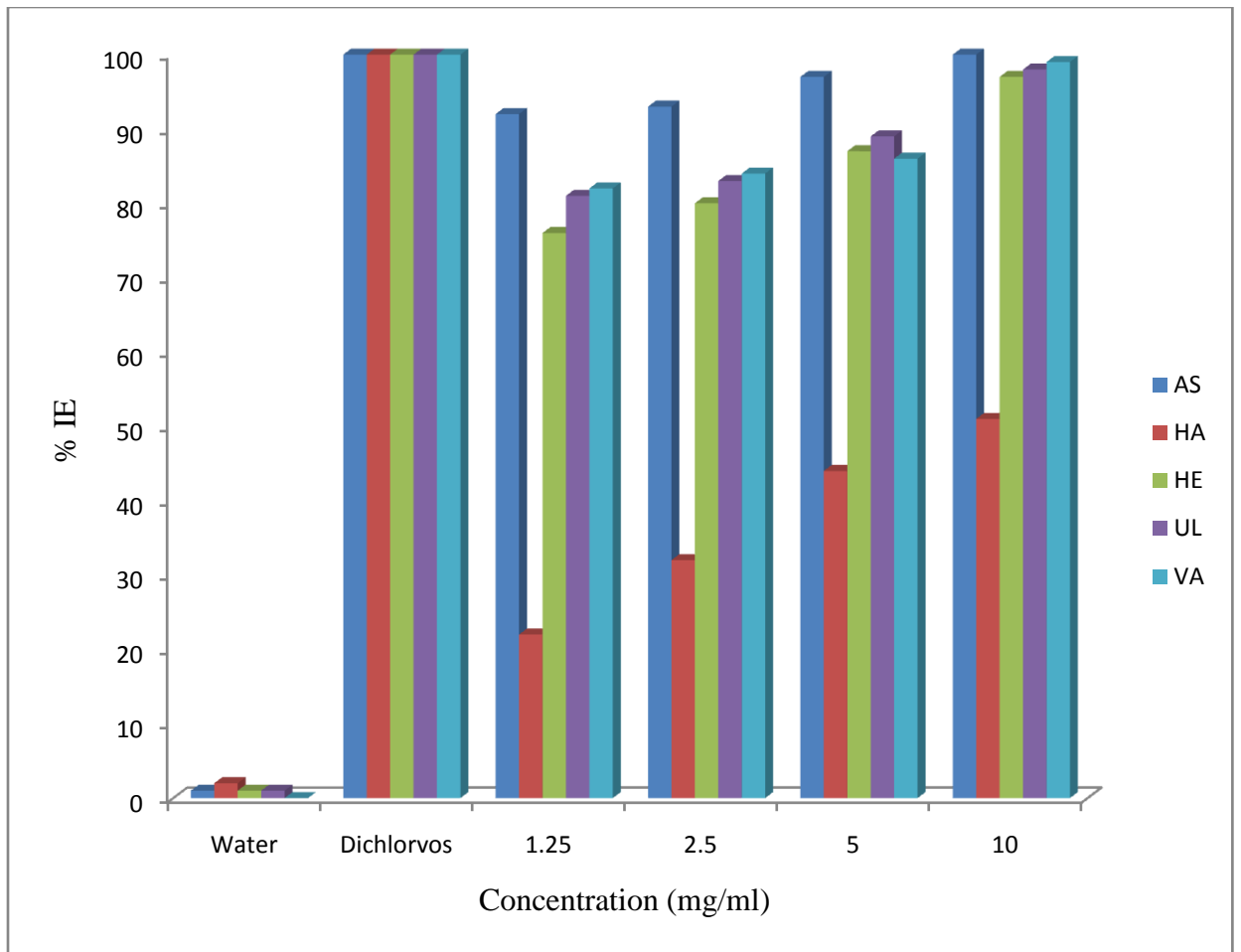


Figure 4.5: Inhibition of adult emergence in *Culex quinquefasciatus* by ethyl acetate leaf fractions of selected ethnomedicinal plants

4.18 Number of Life Stages of Emergent Adults of *Culex quinquefasciatus* reared in Water (control) and Ethylacetate Leaf Fraction of *A. senegalensis*

The emergent of adults *Culex quinquefasciatus* exposed to untreated (control) and ethyl acetate fraction of *A. senegalensis* is shown in Figure 4.6. The number of eggs per raft in control was 101 and it was higher than the one recorded in Ethyl acetate fraction (70). All the eggs obtained in control hatched successfully into first larval instar; the number reduced from 70 to 52 in ethyl acetate fraction. As the stages of development progressed from one instar larvae to another, there was reduction in number of larvae. It was obtained as 46, 36 and 25 at L2, L3 and L4, respectively. There were 18 numbers of larvae that emerged into pupae compared to 100% recorded from control. The female to male ratio of the control emerged adults of *Culex quinquefasciatus* was 1 to 1 respectively, while Ethyl acetate fraction was 3 to 7 also for female to male ratio, accordingly.

4.19 Duration of Life Stages of Emergent Adults of *Culex quinquefasciatus* reared in Water and Ethyl acetate Leaf Fraction of *A. senegalensis*

The number of days taken by *Culex quinquefasciatus* to transform from one developmental stage to another is shown in Figure 4.7. There was variation in number of days taken by *Culex quinquefasciatus* to emerge. It took a shorter period for control group to emerge in all the developmental stages compared to ethyl acetate fraction of *A. senegalensis*. It took 2 days for eggs in the control to emerge into L1 compared to 4 days recorded by ethyl acetate. The transformation of L1 to L4 for Ethyl acetate fraction of *A. senegalensis* was 3 days each compared to 1.8 days (43.2 hours) at L1, L2 and L3 and 1.5 day for L4 taken by the control. Pupation in ethyl acetate group was found to be 4 days compared to 1.8 days obtained in control.

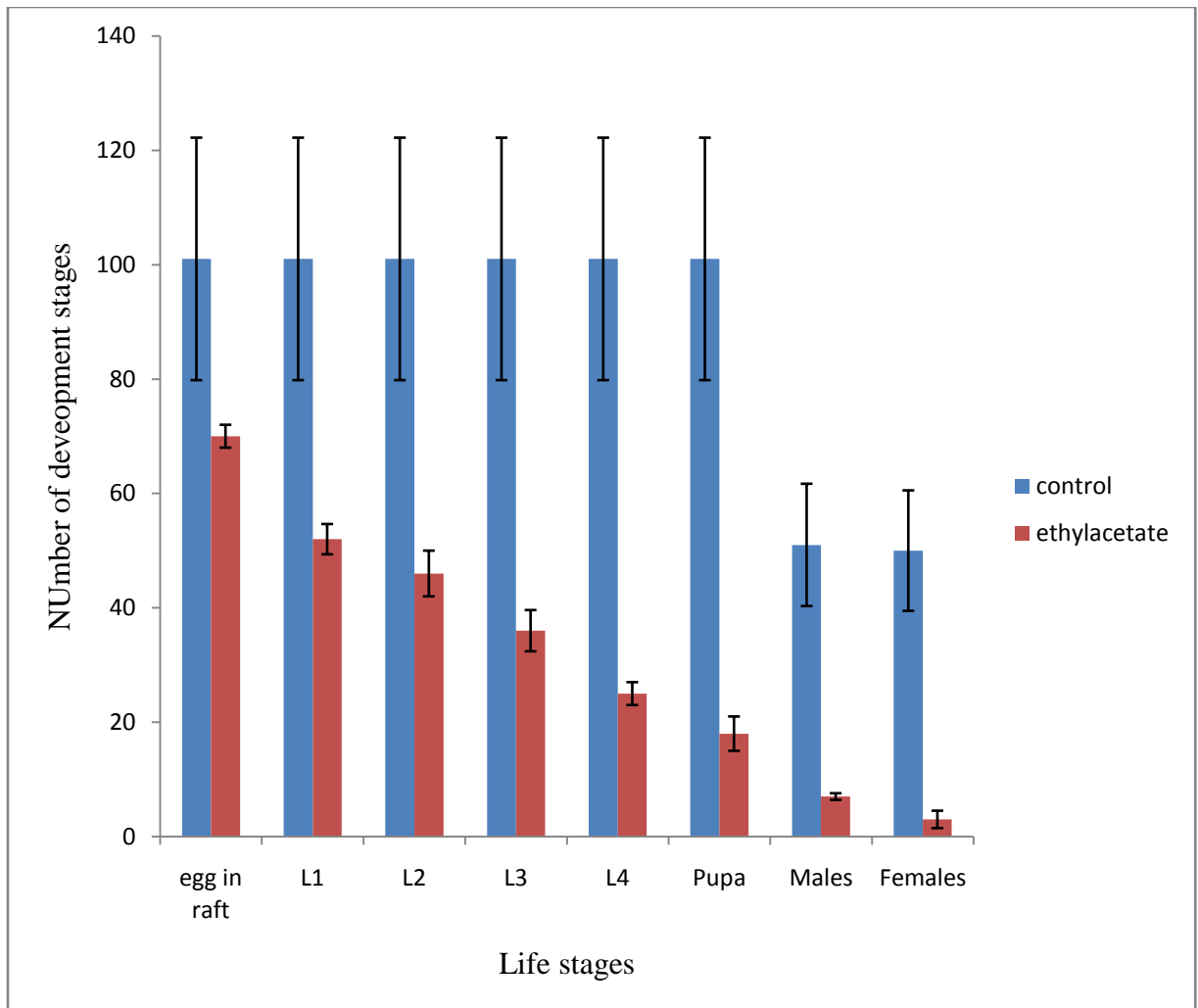


Figure 4.6: Effect of Ethylacetate Leaf Extract of *A. senegalensis* on the Oviposition, Larval and Adult Development of *Culex quinquefasciatus*

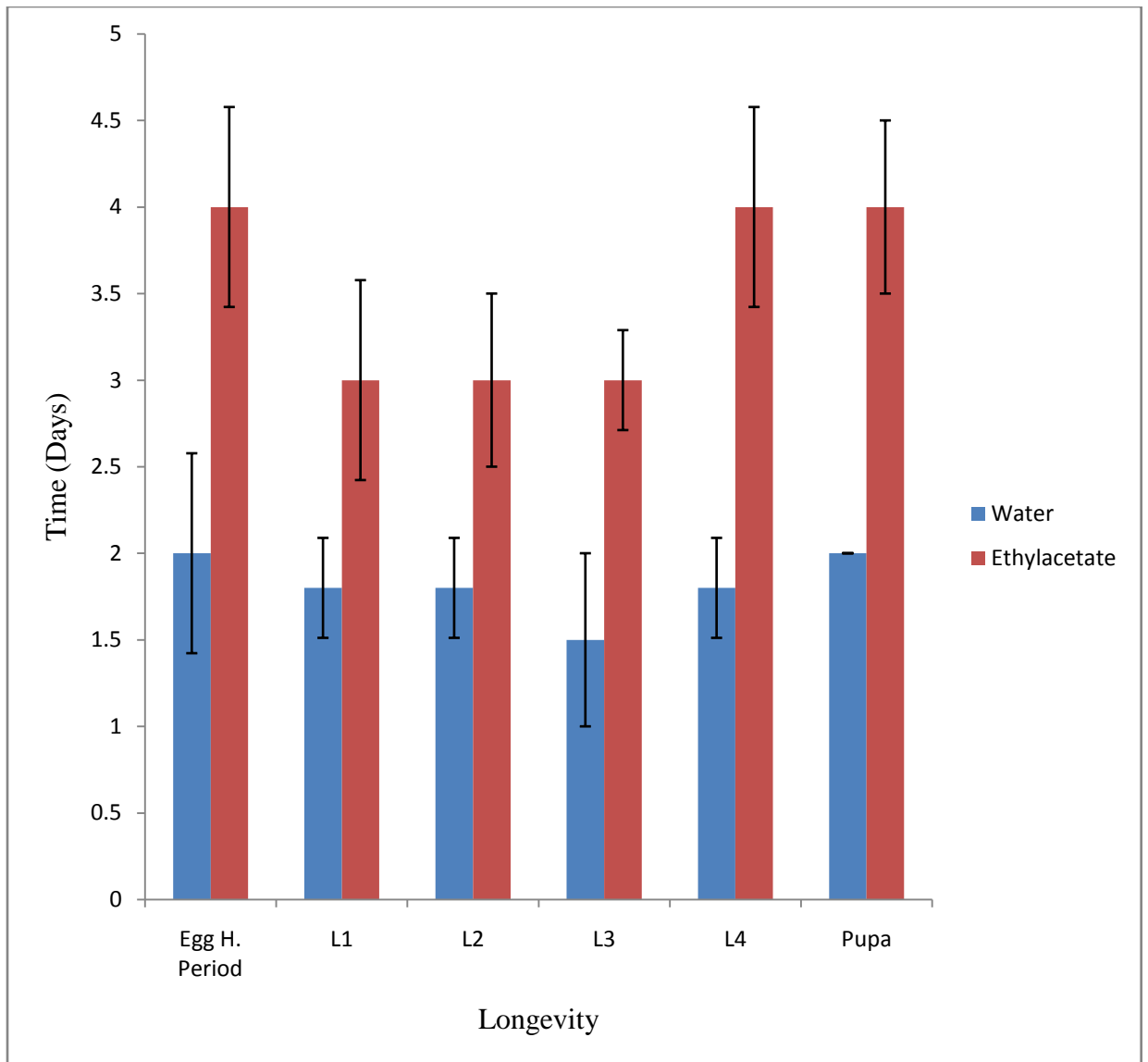


Figure 4.7: Duration of life stages of emergent adults of *Culex quinquefasciatus* reared in water and ethylacetate leaf fraction of *A. senegalensis*

4.20 Morphometric Measurements of Life Stages of Emergent Adults of *Culex quinquefasciatus* Ethyl Acetate Leaf fraction of *A. senegalensis*

The morphometric measurements of life stages of emergent adults of *Culex quinquefasciatus* exposed to ethyl acetate leaf fraction of *A. senegalensis* is shown in Figure 4.8. The largest and the smallest raft of *Culex quinquefasciatus* in control (water) were 263 and 256 ocular units. There was remarkable difference between largest and the smallest egg rafts produced by *Culex quinquefasciatus* exposed to ethyl acetate fraction of *A. senegalensis*; this was 136 and 92 ocular units for large and small raft, respectively. There was progressive increase in length of larvae from L1 to L4 in both control (water) and ethyl acetate fraction. The lengths of L1, L2, L3 and L4 were recorded as 98, 210, 269 and 493 ocular units, respectively for the control and 68, 126, 183 and 281 ocular units for L1, L2, L3 and L4 for the ethyl acetate fraction, respectively. There was decrease in pupal length of the control (173) and ethyl acetate fraction (104) ocular units.

4.21 Sex Ratio of Emerged Adults of *Culex quinquefasciatus* Exposed to Ethyl Acetate Fraction of *A. senegalensis*

The reproductive ratios of emergent adults of *Culex quinquefasciatus* exposed to water (control) and ethyl acetate are shown in Figure 4.9. The ratio of number of egg rafts laid per female *Culex quinquefasciatus* in the Control group and ethyl acetate group of *A. senegalensis* was found to be 3 to 1. The percentage of female to male in the Control was 49% and 51% (1:1), respectively; while in ethyl acetate fraction group, it was 32% for female and 68% for male (1:2).

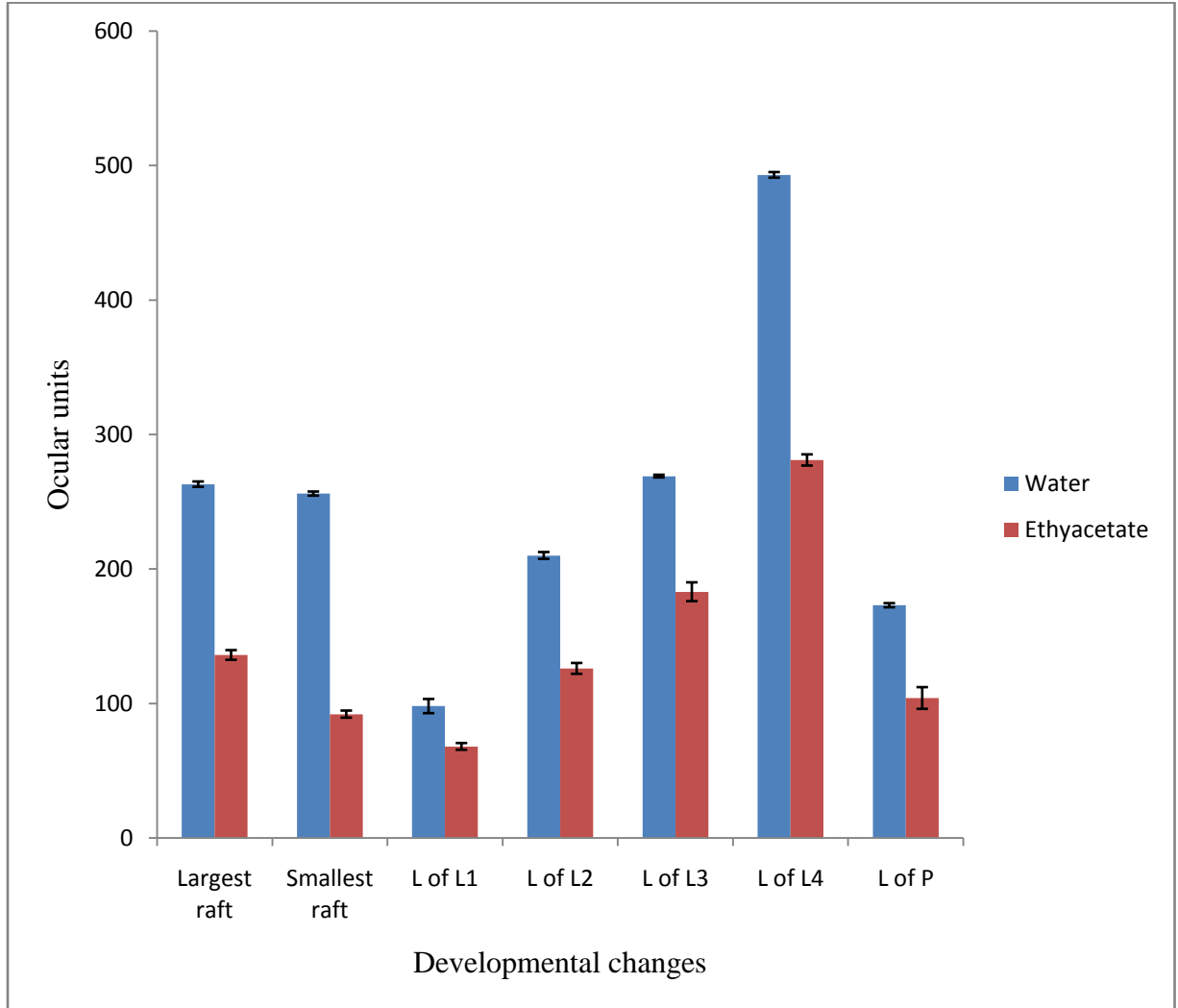


Figure 4.8: Morphometric Changes of life stages of *Culex quinquefasciatus* exposed to ethylacetate leaf fraction of *A. senegalensis*

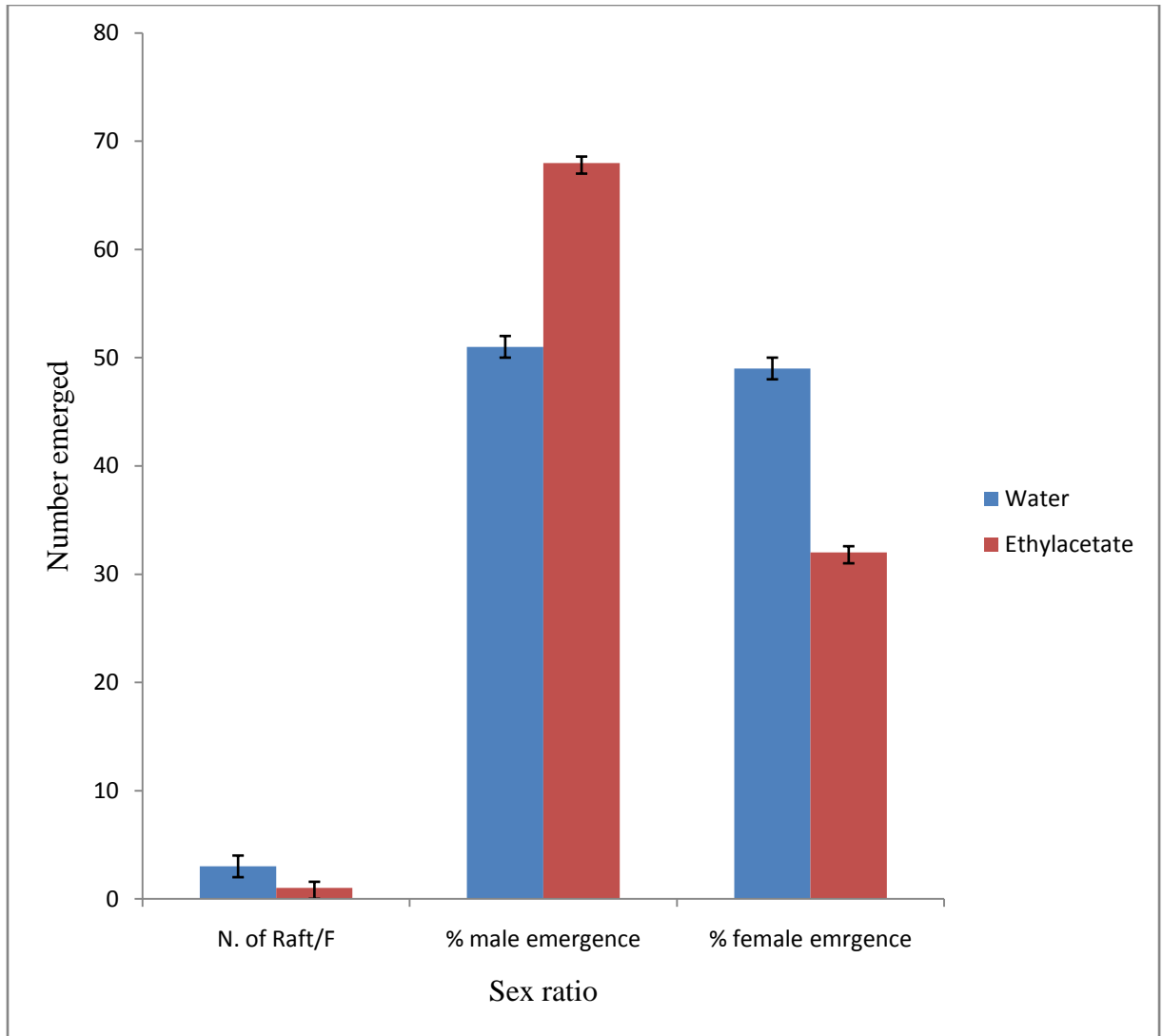


Figure 4.9: Sex ratio of emerged *Culex quinquefasciatus* exposed to ethylacetate leaf fraction of *A. senegalensis*

4.22 Acute Toxicity of Compound ‘YU’ of Ethyl Acetate Leaf Fraction of *A. senegalensis*

The acute toxicity of compound ‘YU’(Ergosterol)on *Culex quinquefasciatus* larvae is shown in Table 4.11. There was significant difference ($p < 0.05$) in larval mortality among the concentrations. There was 100% mortality in dichlorvos, while water did not record any mortality. Mortality at experimental concentrations increased with increase in dose. The mortality at dose 1000 $\mu\text{g/ml}$ (8.00) was significantly ($p < 0.05$) highest followed by 500 $\mu\text{g/ml}$ (6.00), 250 $\mu\text{g/ml}$ (3.25) and the least was at 125 $\mu\text{g/ml}$ (1.75). Percentage mortality ranged from 0.00 in untreated water (negative control) to 100% in dichlorvos (positive control).

4.23 The ^{13}C NMR Spectrum of Compound Ergosterol

The results of NMR analysis of compound “YU” are shown in Table 4.12. The compound was identified as ergosterol, a sterol, which is commonly found in a mixture with stigmasterol, sitosterol and campesterol (Figure 4.10).Ergosterol was obtained as a colourless amorphous powder.The ^{13}C NMR spectrum of the compound exhibited 28 carbon signals for steroidal molecules and 6 carbon signals for ethyl and hydroxyl groups (Table 4.12).

Table 4.12: Acute Toxicity of Compound (Ergosterol) in ethyl acetate leaf fraction of *A. senegalensis*

Concentrations ($\mu\text{g/mL}$)	Mortality (Mean \pm SE)	% Mortality
Control (Water)	0.00 ^f \pm 0.00	0.00
Control (0.1% Dichlorvos)	10.00 ^a \pm 0.00	100.00
125	1.75 ^e \pm 0.48	18.00
250	3.25 ^d \pm 0.48	33.00
500	6.00 ^c \pm 0.41	60.00
1000	8.00 ^b \pm 0.41	80.00

Means with different superscript per column are significantly different ($p < 0.05$).

EDMAN_MSR_L_YU
 Sample Name:
 EDMAN_MSR_L_YU
 Data Collected on:
 Agilent-NMR-vnmr400
 Archive directory:
 /home/vnmr1/vnmr400/data
 Sample directory:
 EDMAN_MSR_L_YU_20160408_01
 FIDFile: PROTON_01
 Pulse Sequence: PROTON (s2pul)
 Solvent: cdcl3
 Data collected on: Apr 8 2016
 Temp. 25.0 C / 298.1 K
 Operator: vnmr1
 Relax. delay 1.000 sec
 Pulse 45.0 Degrees
 Acq. time 2.556 sec
 Width 6410.3 Hz
 8 repetitions
 OBSERVE H1, 399.9252270 MHz
 DATA PROCESSING
 FT size 32768
 Total time 0 min 28 sec

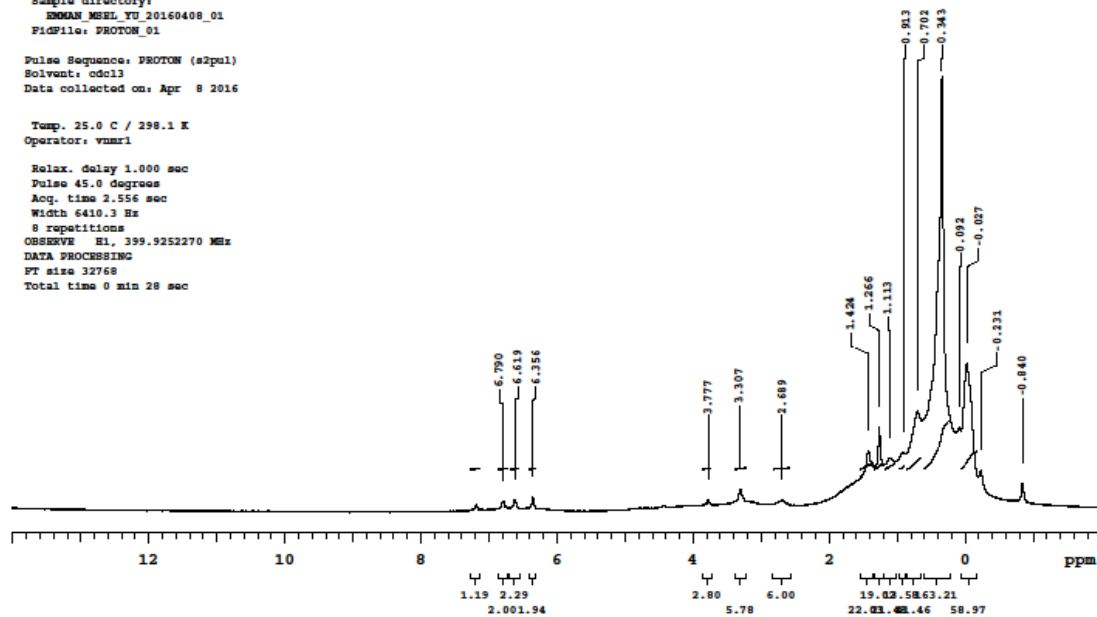


Figure 4.10: ^1H NMR spectrum of compound

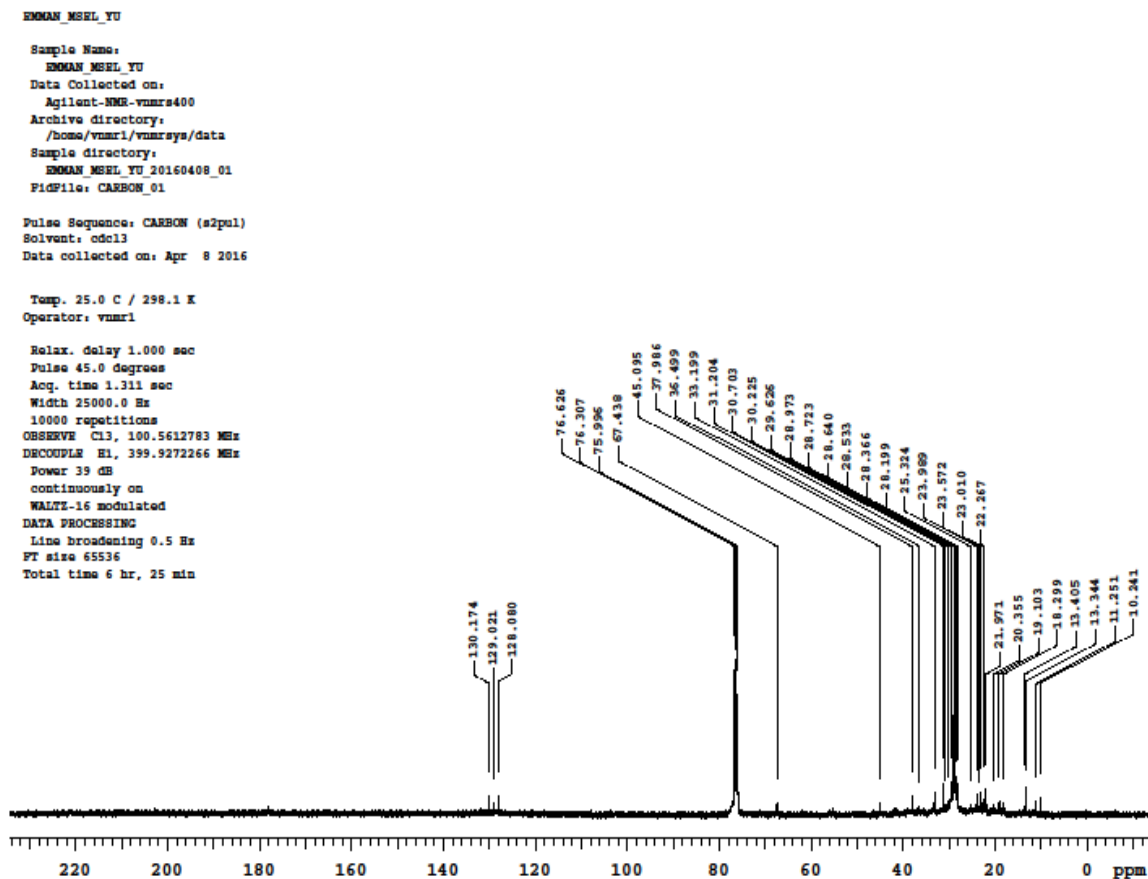
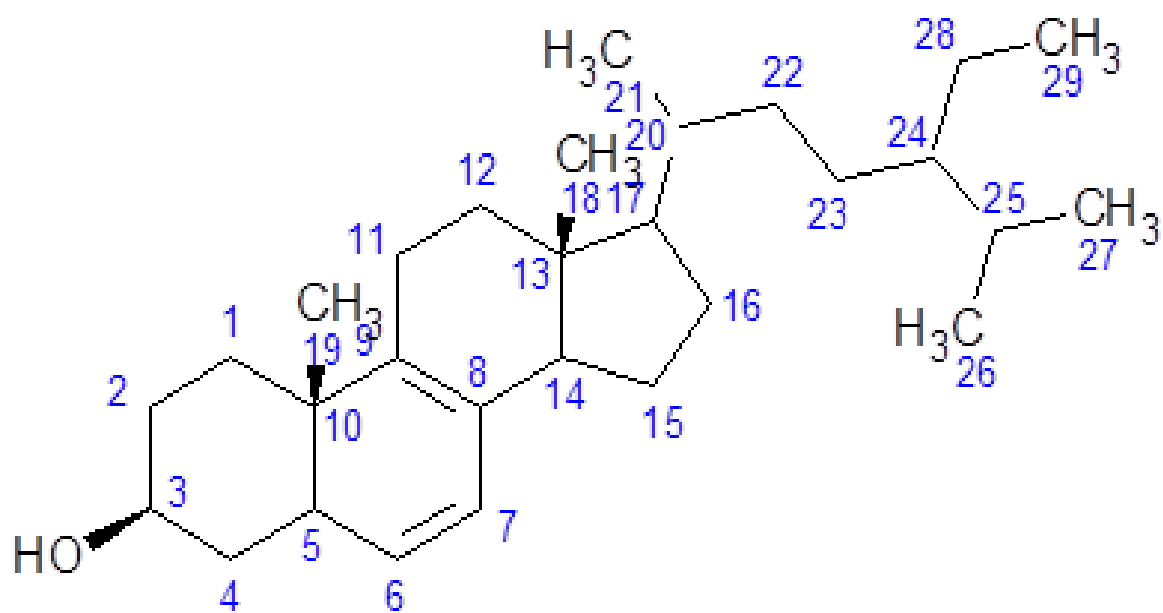


Figure 4.11: ^{13}C Carbon spectrum of the compound, ergosterol

Table 4.13: ^{13}C NMR Spectral Values of the Compound, Ergosterolo

S/N	Position	Ergosterol (ppm)	Literature Parwaiz <i>et al.</i> (2010) (ppm)
1	C1	28.53	31.30
2	C2	28.64	31.50
3	C3	67.43	70.90
4	C4	30.70	37.67
5	C5	31.20	42.79
6	C6	128.08	128.87
7	C7	129.02	129.06
8	C8	136.17	136.44
9	C9	130.17	136.75
10	C10	30.23	37.65
11	C11	23.01	21.42
12	C12	29.00	36.50
13	C13	33.20	43.56
14	C14	37.99	54.33
15	C15	23.99	23.48
16	C16	28.21	27.92
17	C17	45.10	56.34
18	C18	18.31	12.32
19	C19	19.10	16.19
20	C20	29.63	36.71
21	C21	21.97	18.94
22	C22	28.72	34.00
23	C23	25.32	26.47
24	C24	36.50	46.03
25	C25	28.37	28.92
26	C26	20.35	18.25
27	C27	22.27	20.25
28	C28	23.57	23.30
29	C29	12.31	13.41



24-ethylcholesta-6,8-dien-3-ol (ergosterol)

Figure 4.12: Chemical structure of the compound, ergosterol

4.24 Separation of Chemical Constituents

In this study, the acute toxicities of aqueous and ethanolic extracts of the leaves of *Annona senegalensis* were tested on *Culex quinquefasciatus* larvae. The ethanolic extract had higher activity compared to the aqueous extract. Hence, bioassay-guided fractionation of *A. senegalensis* crude ethanolic extract was conducted by partitioning and later by column chromatography (Plates VIII-XVI). Aqueous, n-butanol, n-hexane and ethyl acetate fractions were obtained. The ethyl acetate fraction had the highest activity, hence, ethyl acetate fractions of *H. asper*, *H. esculentus*, *U. lobata* and *V. amygdalina* were obtained for comparative analysis. The ethyl acetate fraction of *A. senegalensis* retained its highest activity; it was used to determine effects on survival rates and fecundity.

The Thin Layer Chromatography (TLC) of *A. senegalensis* was carried out with various spraying agents (TLC of ethyl acetate spraying agent) and solvent systems (TLC of ethyl acetate). The n-hexane/ethyl acetate, v/v 60/40, was retained. After eluting on Column, a preparative TLC was carried out on the ethyl acetate fraction of *Annona senegalensis* fraction to compare with the crude extract. Nine subfractions were identified by characteristic colourations. Subfraction 9 did not give any clear spots so it was discarded (Control of ethyl acetate of *A. senegalensis*). Subfractions that gave similar spots were pooled together into compounds (A₁ and A₂), B (B₁ and B₂) and C.

Compound B was identified as a pure compound. It was colourless and dissolved in chloroform. It gave R_f value of 0.57 (chromatogram of compound). Acute toxicity of was carried out and 10.00mg of the compound was sent for NMR analysis for identification.

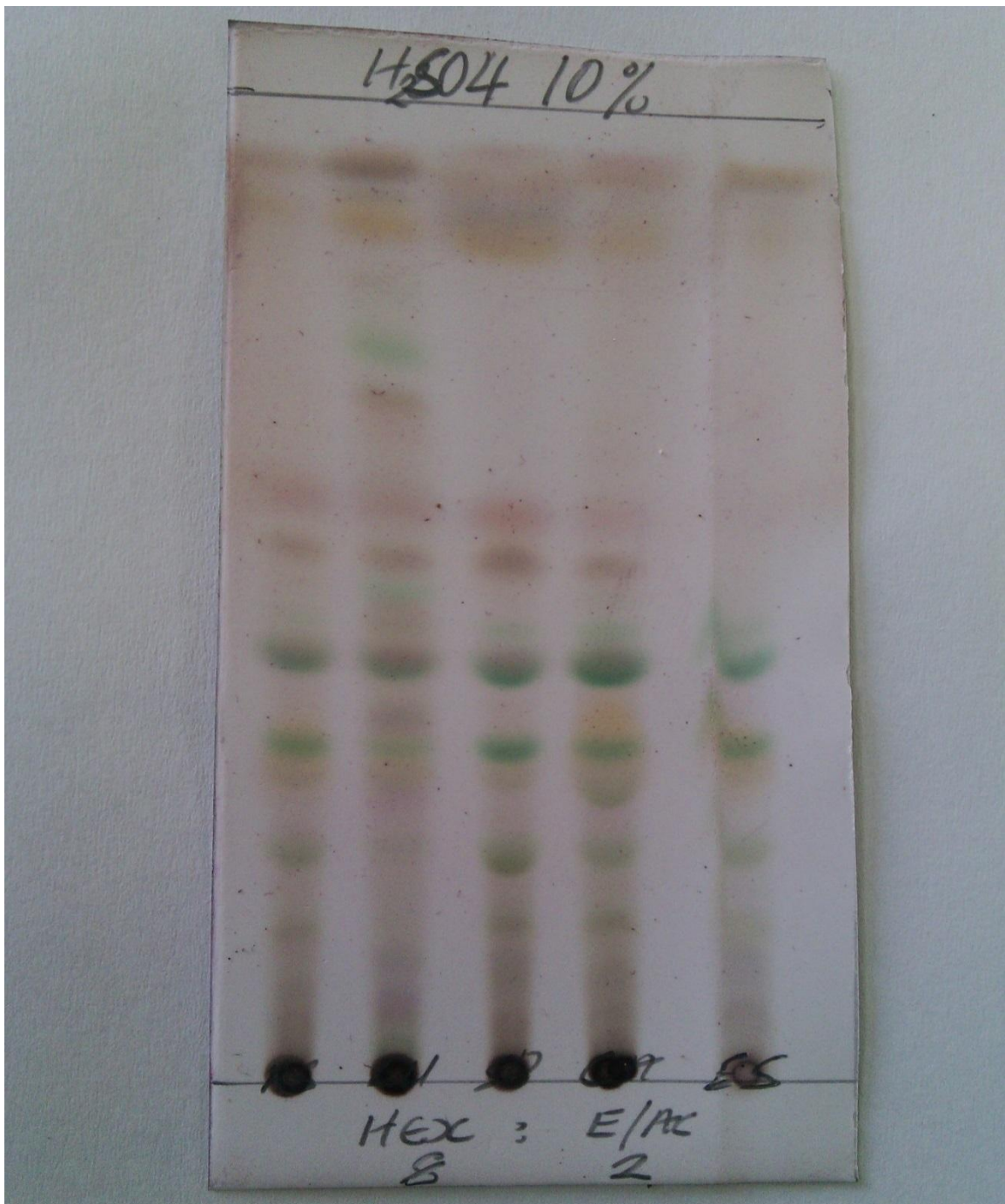


Plate VIII: Thin layer chromatography leaf extracts of *Hibiscus* sprayed with sulphuric acid

Notes: The plate shows spots of the samples at various positions at the bottom of the chromatogram. Sample showed same pattern of spreading along the column.

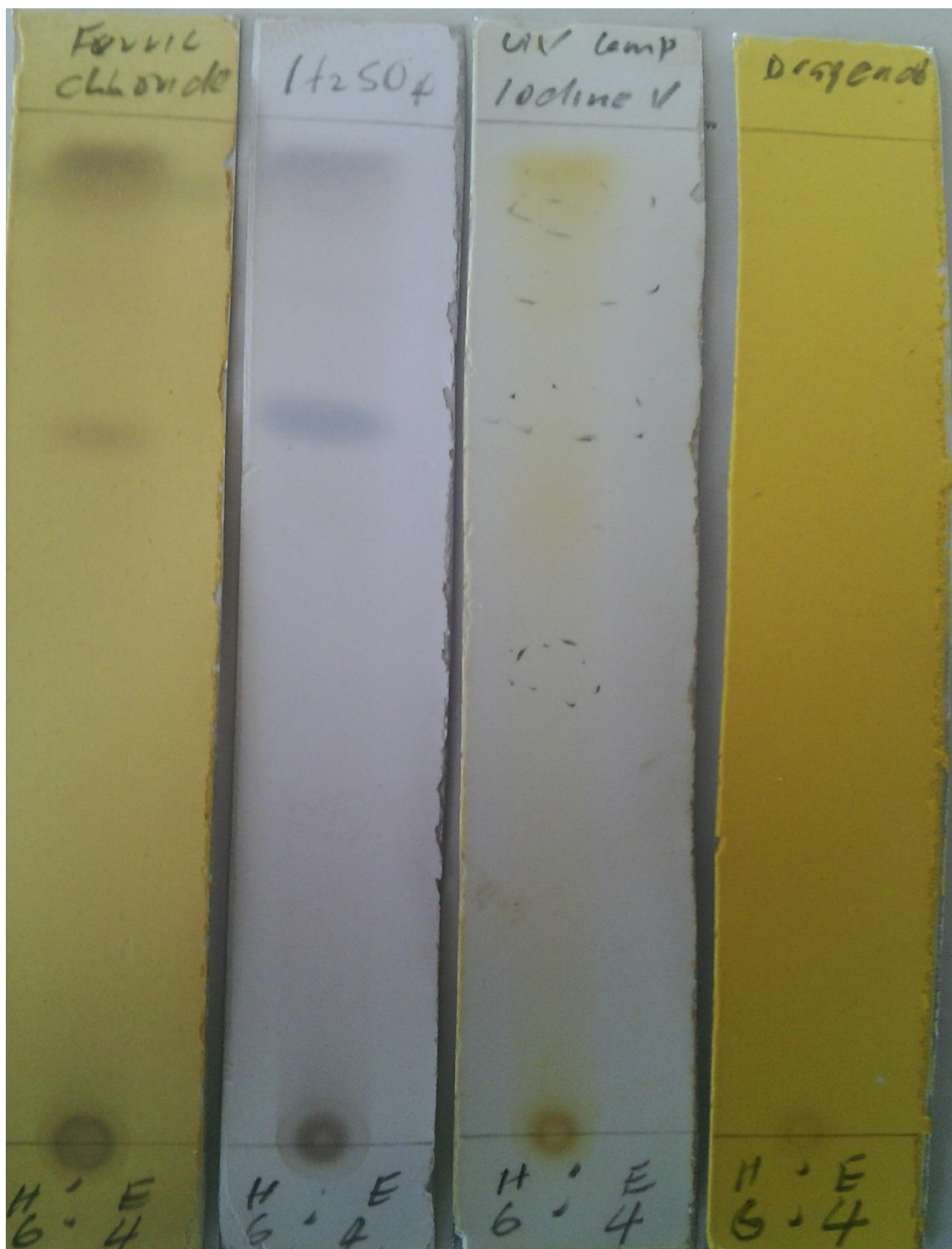


Plate IX: Ethyl acetate leaf fraction of *Annona senegalensis* in various spraying agents and UV light

Notes: *Annona senegalensis* leaf extracts was spotted on different chromatogram, only H.E/6.4 showed spreading along the column with H.E/6.2 spreading around its perimeter.

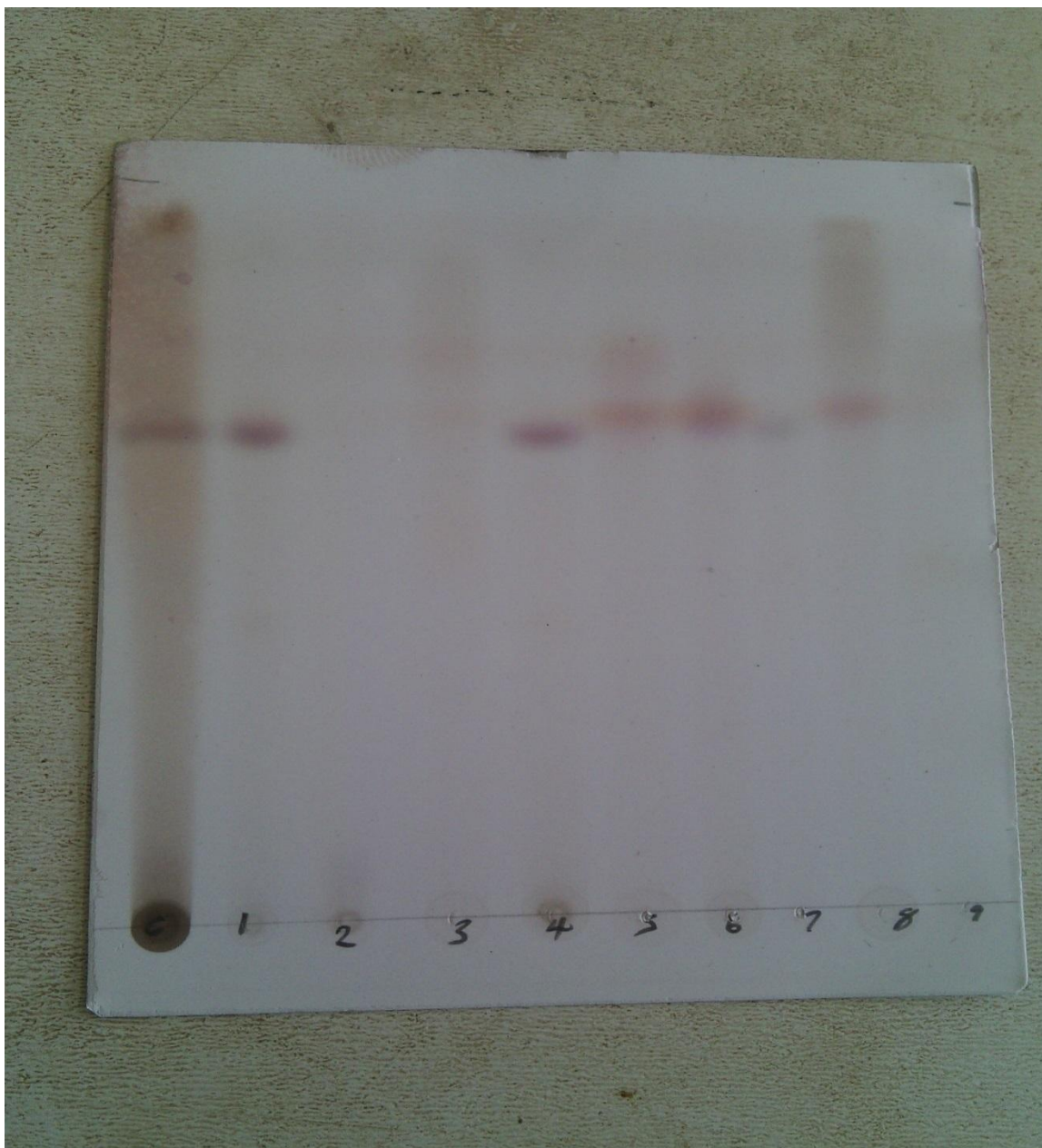


Plate X: Chromatogram of ethyl acetate leaf sub-fractions of *Annona senegalensis*

Notes: Ethyl acetate fraction of *Annona senegalensis* spread up from all the spot and form a visible spot mid-way.

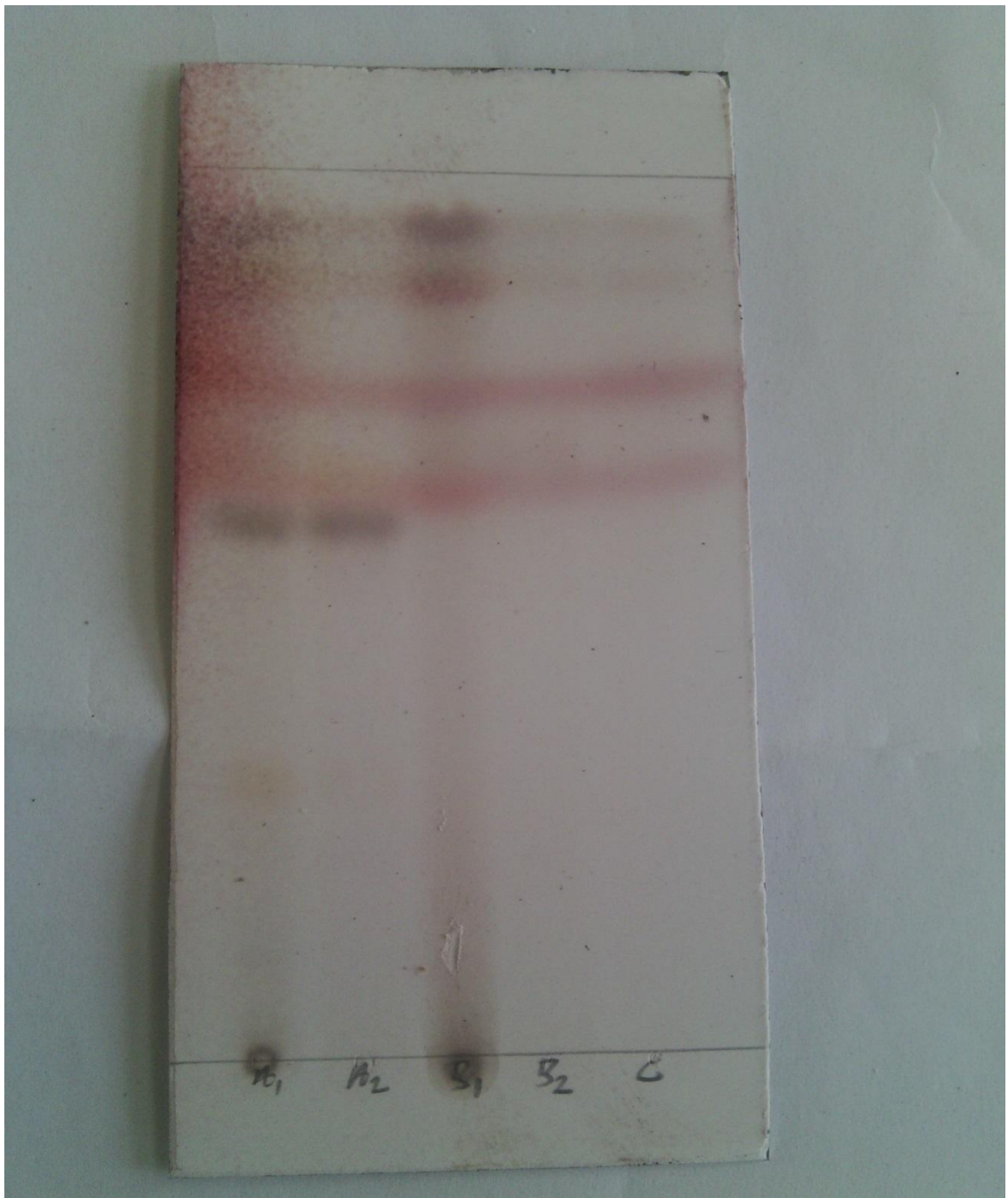


Plate XI: Chromatogram of pooled ethyl acetate leaf subfractions of *Annona senegalensis*

Notes: Subfraction of ethyl acetate of *Annona senegalensis* formed a thick horizontal on top of the chromatogram with B1 visible from the bottom to the top.

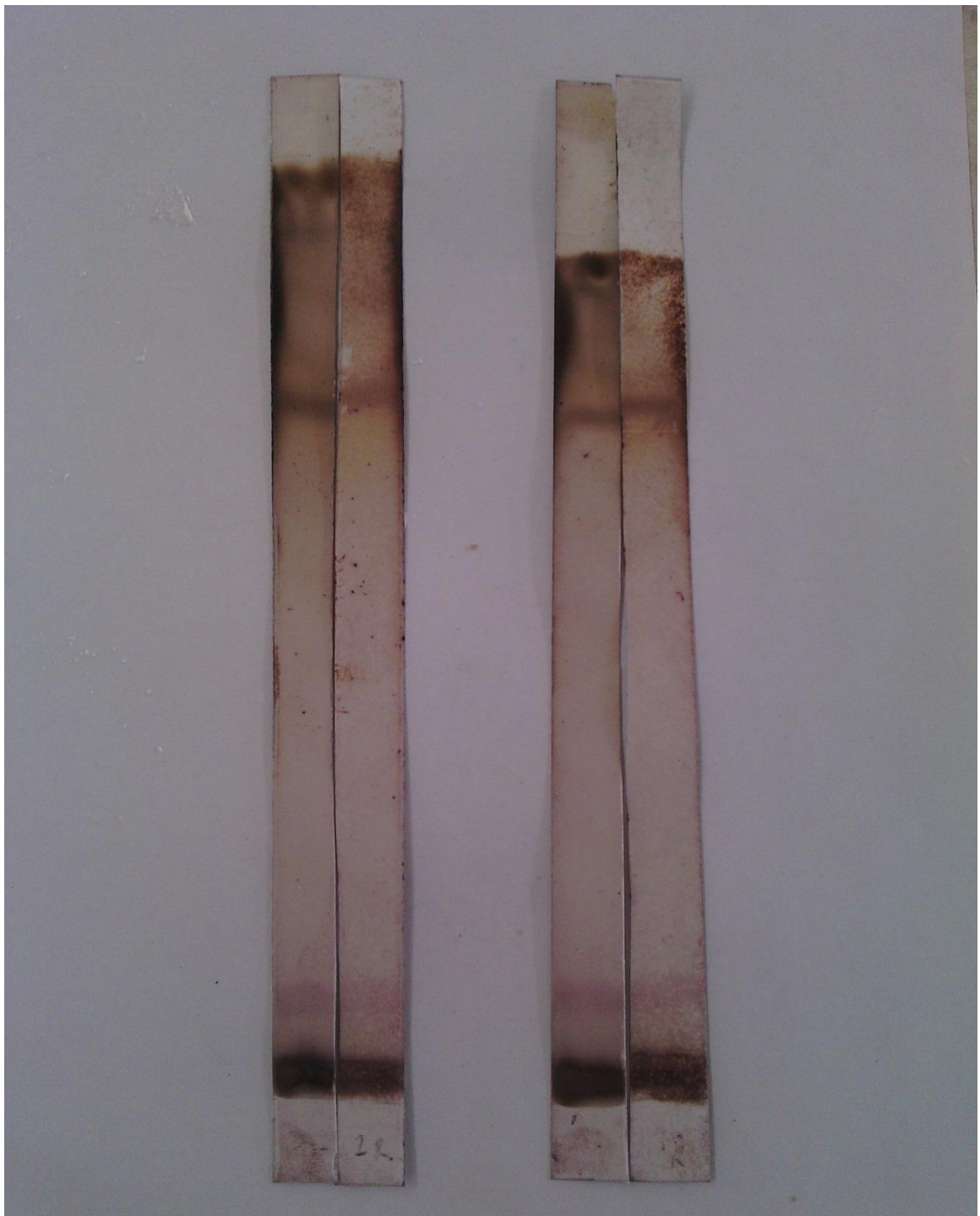


Plate XII: Preparative chromatogram of ethyl acetate leaf fractions of *Annona senegalensis*

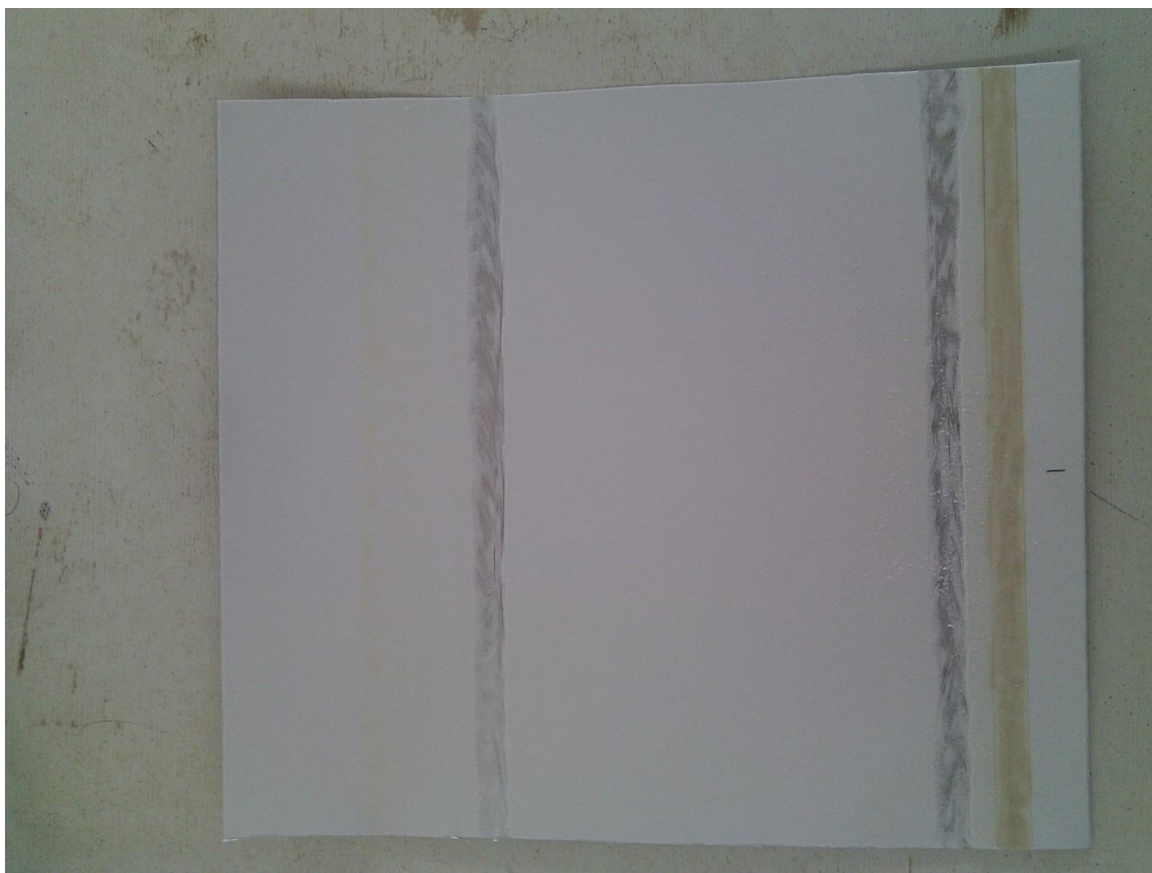


Plate XIII: Scratched preparative chromatogram of ethyl acetate leaf fraction of *Annona senegalensis*

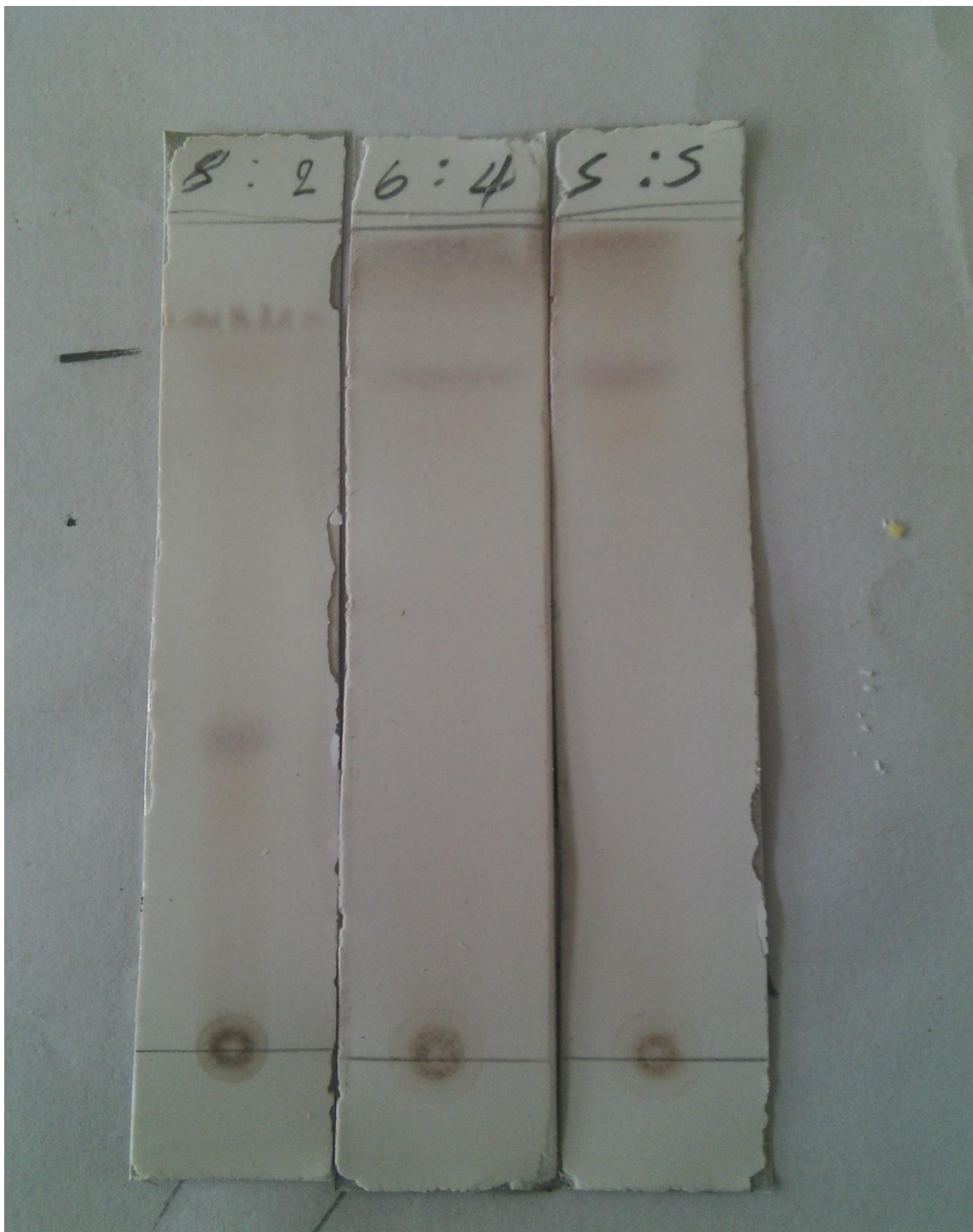


Plate XIV: Ethyl acetate leaf fraction of *Annona senegalensis*

Notes: Ethyl acetate fraction of *A. senegalensis* moved from the bottom where it was spotted to the top on all the three chromatogram sheets.

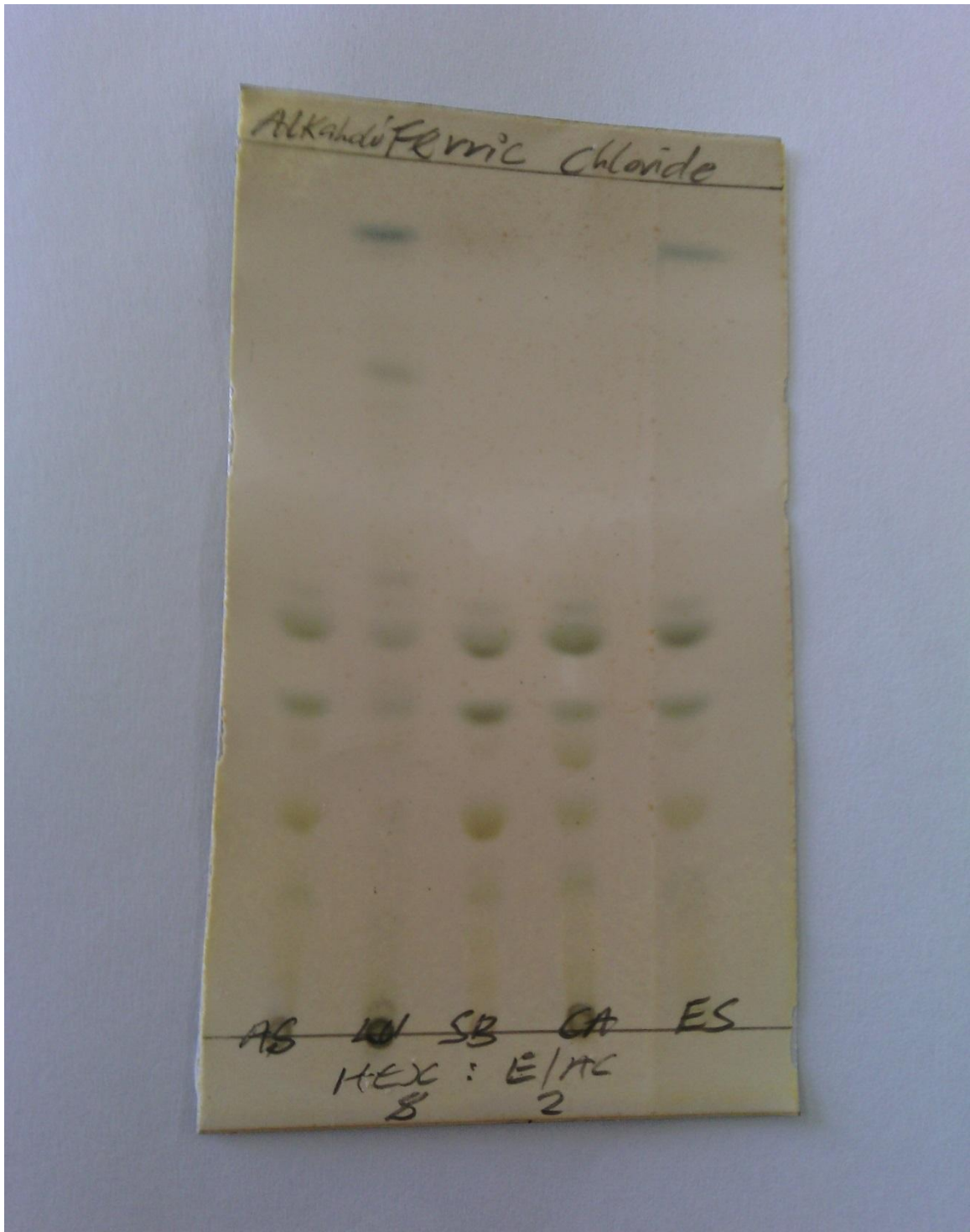


Plate XV: Thin layer chromatography leaf extracts of *Hibiscus* sprayed with ferric chloride

Notes: Leaf extract of *Hibiscus* spreads up from all the spots and formed a uniform at five different spot. Spot of WI and ES moved to the top of the chromatogram

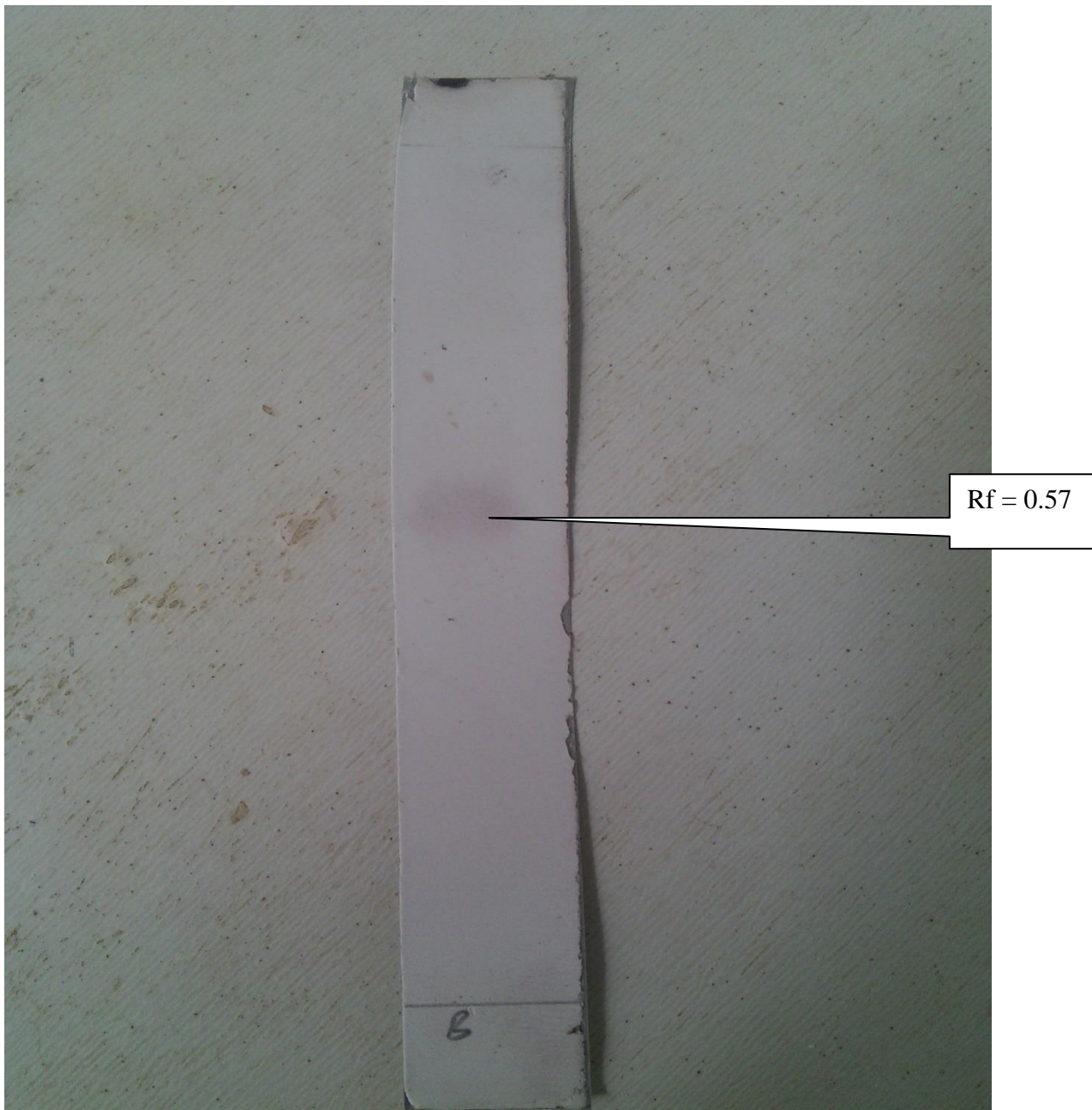


Plate XVI: Chromatogram of compound of ethyl acetate leaf fraction of *Annona senegalensi*

Notes: Same compound of ethyl acetate leaf fraction formed a faint spot at the centre of chromatogram ($R_f = 0.57$)

CHAPTER FIVE

5.0

DISCUSSION

In this study, some selected plants have exhibited cidal effect on the larvae of *Culex*. There was presence of carbohydrate in all the preliminary plants screened for larvicidal activity. This may be as a result of carbohydrate being the primary source of energy to both plants and animals that is required for daily activity. Anthraquinone was not found in all the *Hibiscus* species and *Urena lobata* but was present in *Vernonia amygdalina* and *Annona senegalensis*. Anthraquinone is not commonly associated with shrubs, hence its absence in *Hibiscus* species tested and *Urena lobata* (Rajeseekariah, 1999). *Vernonia amygdalina* and *Annona senegalensis* are tree plants and anthraquinone is associated with them. Different plants may synthesize and accumulate different compounds or different amounts of a particular compound due to their differential gene expression, which in turn, affects antioxidant activities and other biological properties of the plant extracts (Randhira *et al.*, 2004).

Combined anthracenes were absent in all the screened plants except *V. amygdalina* where they were found in minute quantities. The presence of these phytochemicals in *V. amygdalina* could be due to the state of the plants during collection, older tree tend to have more phytochemicals than the younger ones. The phytochemicals such as unsaturated triterpenes, unsaturated sterols, cardiac glycosides, saponins and tannins were found in different proportions in all the screened ethnobotanical plants in this study. The aforementioned phytoconstituents are known to form the basic constituents of most plants. Flavonoids were found in higher quantity in *Annona senegalensis* but they were absent in *U. lobata* and *V. amygdalina*. The presence of flavonoids in higher quantity in

A. senegalensis is an indication of a strong mosquitocidal activity (Layet *et al.*, 2014), which was observed in the larval mortality caused by *Culex quinquefasciatus* of this study.

Alkaloids were only found in minute quantity (+) in *H. cannabinus* and *A. senegalensis*. This may be as a result of contribution of related environmental factor in which the plants was grown. Studies have confirmed that the amount and composition of phytoconstituents compounds differ from one plants to another and is found at the sub-cellular level and within plant tissues as well (Jaffery *et al.*, 2003; Rafat *et al.*, 2010). Moreover, many flavonoids and terpenoids are potent antioxidants, with anti-inflammatory, antibacterial, antiviral, anticancer and larvicidal properties (Layet *et al.*, 2014). These plants also contain steroids, which are known to mediate cardiogenic activities and possess insecticidal and antimicrobial properties, while tannins, which were also found in varied concentrations (+, ++, +++) these plants, are known to possess general antimicrobial and antioxidant activities (Rievere *et al.*, 2009).

The bioassay of the eight ethnobotanical plants tested in this study recorded significant ($p < 0.05$) different mortality in all the concentrations tested. The larval mortality in the extracts of *A. senegalensis*, *H. cannabinus* and *H. lunariifolius* did not exhibit dose-dependent mortality response. This might be due to homogeneous mixture of active ingredients responsible for a kill, such that small quantity of extracts may cause mortality even at lower doses. Larval mortality in plant extract of *H. asper* increased with increase in concentration in which significant difference ($p < 0.05$) was obtained at the highest dose. Similar trend was observed in larval mortality in *H. esculentus* extract but higher larval mortality was recorded at lower doses compared to that in the former. Larval

mortality at *H. sabdariffa*, *U. lobata* and *V. amygdalina* exhibited dose dependent activity. The dose dependent activity recorded in this group of plants' extracts could be as a result of few larvicidal ingredients present in the plants which require large quantity to perform its full potentials. Hence, the increase in larval mortality with increase in concentrations of the plant extracts observed. This is in accordance with the report of Senthilnathan (2007) which stated that the higher the leaf extract of *Eucalyptustereticornis* oil with increased doses on *Anophelesstephensi*, the greater the larvicidal effect. Senthilnathan also observed that first and second instar larvae were most susceptible to all treatments.

Saponins and alkaloids have been reported by Mousumi *et al.* (2013) to be responsible for the toxicity of seed coat of *Cassia sophera* on all instar larvae of *Culexquinquefasciatus*. According to a research, tannins and alkaloids in *Pistiastratiotes*; tannins, alkaloids and steroid glycosides in *Typhalatifolia*; tannins, saponins and steroid glycosides in *Leucasmartinicensi*; alkaloids, saponins and tannins in *Cynodondactylon* and saponins and tannins in *Nymphaealotus* have been reported to be responsible for larval toxicity of *Anopheles* mosquitoes (Imam *et al.*, 2013).

In addition, triterpenoids and saponins in chloroform; saponins in hexane, steroids, saponins, tannins and alkaloids in methanol extracts of *Adansoniadigitata* are toxic for *Ae. aegypti* and *Culexquinquefasciatus* larvae (Krishnappa *et al.*, 2012). All the extracts and fractions in the present study contained one or more phytochemical compounds. Therefore, the larvicidal activity might be due to the presence of those phytoconstituents.

There were differences in the LC₅₀ and LC₉₀ of the screened ethnobotanical plants tested against *Culex quinquefasciatus* larvae. *Annona senegalensis* recorded the least LC₅₀ of 0.457mg/ml and LC₉₀ to be 5.202mg/ml. This showed that the extract of *A. senegalensis* was more lethal to mosquito larvae than all the other extracts. This could be due to the presence of all the expected insecticidal phytochemical constituents found in the extracts of *A. senegalensis*. *Hibiscus asper* had the highest LC₅₀ value of 4.032mg/ml and it performed poorly in terms of its larvicidal properties. Flavonoids were not found in the extract of *H. asper* and this might have been responsible for the low mortality observed. *Hibiscus lunariifolius* had the least LC₉₀. There was general low larval mortality across all the concentration of *H. lunariifolius* which could have been responsible by the absence of the effects of active compounds. The LC₅₀ recorded in this study were all higher than the one reported by Nganjiwaet *al.* (2015) to be 6.03ppm for *Balanites* plant which indicated that it was more potent than *Calotropis* methanol leaf extract and *Eucalyptus* methanol leaf extract LC₅₀ which were 6.31 and 6.39 ppm respectively. They further explained that the higher the mortality rate, the lower the LC₅₀ value. It also means that at lower concentration of *Balanites* extract, 50% mortality was recorded at 5.70 ppm compared to 5.75 and 6.03 ppm.

The larvicidal activity of ethanolic and aqueous leaf extracts of *A. senegalensis* both recorded larval mortality at all the concentrations tested. Ethanolic extract performed significantly ($p < 0.05$) better than aqueous extract of *A. senegalensis* at doses 5.00mg/ml and 10.00mg/ml. This could be due to the inability of the aqueous extract to bring out the potent phytochemical constituents that would have caused more mortality. Water being polar, can only extract polar compounds leaving behind most of the non-polar

compounds which are insecticidal in nature and hence, the low mortality observed. Ethanolic extracts on the other hand performed better because of the active larvicidal compounds present. This study is in disagreement with the work of Pirre (2014) in which the aqueous extract caused high larval mortality. It attained larval mortalities of 26.67%, 81.33% and 85.33% at 500, 1000 and 2000 ppm respectively with LC₅₀ value of 761.26 ppm. Even though, his concentrations were higher than the one used this study. The same result had been reported by Naveed and Muhammad (2011) where the aqueous fraction showed lesser efficacy than hexane, chloroform, ethyl acetate, methanol and butanol fractions.

There was decrease in the number of larvae that metamorphosed successfully to adult that were exposed to ethyl acetate fraction of *A. senegalensis*. After 24h of exposure some few larvae survived in dose 10.00mL but did not metamorphose into pupae. The survival rate to adult further decreased in the remaining concentrations to 0.75, 2.00 and 2.50 at doses 5.00mg/ml, 2.50mg/ml and 1.25mg/ml respectively. The low emergence of adults observed in this study might be due to the toxic effect of ethyl acetate fraction of *A. senegalensis* on the developmental stages of *Culex quinquefasciatus*. The proclamation was valid because of the successful emergence of *Culex quinquefasciatus* in control (water) and the 100% mortality in dichorvos (positive control). The inhibition of emergence recorded might be due to the chemical constituents present in the ethyl acetate leaf fraction of *A. senegalensis* that arrested the metabolic activity of the larvae, which could have caused high mortality of larvae. This study is in consonance with the report of Raveen *et al.* (2014) in which the results of their study revealed that hexane flower extract possessed high larvicidal activity when compared to aqueous with LC₅₀ values of

102.54 and 61.11 ppm after 24 and 48 hours respectively. They further observed that larval mortality was dependent on the concentration of flower extract.

The activities of the solvent extracts of *A. senegalensis* tested on *Culex quinquefasciatus* larvae varied significantly ($p < 0.05$) among the solvents. Larval mortality was generally low in all the concentrations of aqueous fraction, but there was a little bit more mortality in n-butanol and n-hexane solvent fractions. The activity of Ethyl acetate fraction was better than the other three fractions mentioned earlier with 93.60% mortality recorded at the highest dose. This may be due to the ability of ethyl acetate to extract potent compound of larvicidal prospects in large amount that was responsible for higher percentage mortality. This is in agreement with the work of Rajkumar *et al.* (2005) who reported the effect of crude extracts of the leaves of *Centella asiatica* to possess larvicidal and adult emergence inhibition activity against the mosquito *Culex quinquefasciatus*. They further stressed that the biological activity of the plant extract might be due to the various compounds, including phenolics, terpenoids, and alkaloids, existing in the plants. These compounds might have jointly or independently contributed to produce larvicidal and adult emergence inhibition activity against *Culex quinquefasciatus*. This study is also in agreement with the study of Vinayagam *et al.* (2008) in which the recorded pupal and adult emergence effect was in a dose dependent manner. Pupation in 5% treatments was very less when compared to 1 and 3%. No mortality occurred among control larvae, 98% of them pupated and successfully developed into normal adults. Pupae arising from the treated larvae were also affected following treatment. Some of the pupae were straight and could not emerge from the pupal case.

There was mortality in all the doses of ethyl acetate of *A. senegalensis*, *H. asper*, *H. esculentus*, *U. lobata* and *V. amygdalina*. The mortality was dose dependent in all the ethyl acetate fractions of the plants. High mortality was obtained in *A. senegalensis* fraction compared to others, while *H. asper* recorded the least.

The effectiveness of ethyl acetate fraction of *A. senegalensis* could be attributed to flavonoids, tannins, saponins, cardiac glycosides, steroids and triterpenes that were found in higher quantity than in other plants. The mentioned phytochemicals are known to be insecticidal in nature in which they can act independently or jointly to exhibit the effect of mortality. Vinayagam *et al.* (2008) obtained 100% mortality which might be due to the chemical constituents present in the leaf extracts that arrested the metabolic activities of the larvae, which caused higher percentage of mortality. They further emphasized that the increase in turbidity at higher concentration might have blocked oxygen supply to the larvae. The varying results were probably due to the differences in levels of toxicity among the insecticidal ingredients of each plant (Monzon *et al.*, 1994).

Histological changes observed in this study are probably due to activities of the plants extracts that led to the damaging effects on the histology of the organs noticed in *Cx. quinquefasciatus*. DeRobertis *et al.* (1965) and Sutter and Raun (1967) made a similar observations on the effect of *Ostrinia nubilalis* who suggested that the enzymatic activity of the vegetative rods was responsible for the disruption of microvilli. The observed histological effects of the ethanolic extract of *A. senegalensis* on the cephalic region, midgut and abdominal parts of *Culex quinquefasciatus* larvae also agreed with the results obtained by Hamouda *et al.* (1996), Hussin and Shoukry (1997) and Assar and El-Sobky

(2003) on *Culex pipiens* who recorded distorted mid-gut of the mosquito. Hamouda *et al.* (1996) stated that the midgut of *Culex pipiens* treated with *Artemisia judaica* was affected, the epithelial layer was vacuolated, swollen cells, masses of cellular material appeared in the lumen and finally the epithelium lost their normal appearance. Also, they found that larvae treated with *Anagallis arvensis* showed a rupture of the cell wall and destruction of the peritrophic membrane. Assar and El-Sobky (2003) observed that the water extract of *Eichhornia crassipes*, revealed drastic effect on larval midgut as the brush border and some of the epithelial cells were apically degenerated after 48 h and after 72 h, most of the epithelial cells completely degenerated and vacuolated.

The pupal survival of *Culex quinquefasciatus* exposed to ethyl acetate fraction of *A. senegalensis*, *H. asper*, *H. esculentus*, *U. lobata* and *V. amygdalina* varied with different concentrations of each plant. Pupal survival was dose dependent for all the ethyl acetate plant extracts. The survival of pupae in *H. asper* extracts was higher than all the plants extracts tested against pupae. The inability of *H. asper* extracts to kill pupae implies that it has weak active pupicidal compounds. *Anona senegalensis* extracts performed better than all the other extracts tested on *Culex quinquefasciatus* pupae. This observation indicated that, several medium polar compounds in the extract are acting synergistically or competitively at the active site that resulted in pupal mortality.

Rajkumar and Jebanesan (2005) recorded a significant pupal mortality by *Centella asiatica* extracts which they attributed that to the biological activity of the plant extracts which might be due to the various compounds, including phenolics, terpenoids, and alkaloids, existing in the plants; these compounds may jointly or independently

contribute to produce larvicidal and pupal emergence inhibition activity against *Culex quinquefasciatus*. Similar study was conducted by Elimam *et al.* (2009) and they reported that aqueous extracts from leaves of *Ricinus communis* showed 50% of pupal emergence inhibition (EI₅₀) to be 374.97 and 1180.32 ppm against 3rd instar larvae of *Anopheles arabiensis* and *Culex quinquefasciatus* and the extract showed oviposition deterrent effect against both species. The larvicidal, growth inhibitor and repellent actions of *Dalbergiasisoo* oil was evaluated against *An. stephensi*, *Ae. aegypti* and *Culex quinquefasciatus* under laboratory conditions and they observed no adult emergence at 4 ml/m² (Ansari *et al.*, 2000). Deformities produced in pupae further reduced adult emergence from 78% in the control to 75% at 6.25 ppm, 72% at 12.5 ppm, 70% at 25 and 50 ppm, and 66% at 100 ppm and the successful adult emergence from eggs treated was 78% in the control, which was reduced to 45%, 40%, 22%, 17%, and 10% at 6.25, 12.5, 25, 50, and 100 ppm, respectively was observed in methanol extract of *Azadirachta indica* on the development and growth index of *Culex quinquefasciatus* (Sharma *et al.*, 2009).

The adult emergence of *Culex quinquefasciatus* exposed to various plants' fractions of *A. senegalensis*, *H. asper*, *H. esculentus*, *U. lobata* and *V. amygdalina* decreased with increase in concentration. Deformities that developed in the body wall of larvae may be attributed to the dechitinizing effect of extract as reported by Saxena and Sumithra (1985). Tabassum *et al.* (1993) observed that phytoextracts affect larval morphology, resulting in pigmentation and alterations in head and abdomen shape.

The aqueous fraction of *Annona senegalensis* exhibited larval, pupal and adult inhibition of *Culex quinquefasciatus*. The inhibitory activity of aqueous fraction of *A. senegalensis* was dose dependent. This could be due to increase in phytoconstituents with increase in concentrations. Adults emerging from physically deformed pupae remained trapped in the pupal eclusion. The n-butanol fraction of *A. senegalensis* maintained dose dependent effects also but it recorded high inhibitory activity all the concentrations compared to aqueous fraction. However, n- hexane fraction is better than n-butanol fraction This is probably because of its non-polar nature that can extract non-polar compounds from the plant sample which was able to kill more larvae, inhibited higher pupa and adult from emerging. The ethyl acetate fraction *A. senegalensis* performed better than the other three fractions. The inhibitory activity was also dose dependent with no adult emergence in dose 10.00mg/ml. Similar studies have been carried out and these were their findings: Murty *et al.* (1997) have reported significant inhibition of adult emergence in *Culex quinquefasciatus* after treatment with *Polyalthialongifolia* leaf extract. Nathan *et al.* (2005) considered pure limonoids of neem seed, testing for biological, larvicidal, pupicidal, adulticidal, and antiovipositional activity on *An. stephensi* and the larval mortality was dose-dependent with the highest dose of 1 ppm azadirachtin evoking almost 100% mortality, affecting pupicidal and adulticidal activity and significantly decreased fecundity and longevity of *An. stephensi*. Choochote *et al.* (2005) also reported that the adulticidal activity of the hexane-extracted *Curcumaaromatica* (LC₅₀: 1.60 microg/ mg female) was found to be slightly more effective against female *Ae. aegypti* than volatile oil (LC₅₀: 2.86 microg/mg female). The hexane extracts obtained from leaves of *Eucalyptuscitriodoraby* Singh *et al.* (2007) tested at lowest concentration viz. 10 ppm,

73% larvae of *An. stephensi* failed to emerge as adult mosquito, while in *Culex quinquefasciatus* and *Ae. aegypti* only 10% and 6% larvae failed to emerge. Wiesman and Chapagain (2006) reported that the high total saponins compound isolated from methanol extracts of fruit mesocarp of *Balanites aegyptiaca* showed inhibition of 50% of the larval population from emerging into adults (EC_{50} at 0.0014% (w/v) against *Ae. aegypti*. The neem formulation, Neem Azal produced an overall mortality or inhibition of emergence of 90% (EI_{90} , when 3rd instar larvae were treated) at 0.046, 0.208 and 0.866 ppm in *An. stephensi*, *Culex quinquefasciatus* and *Ae. aegypti*, respectively (Gunasekaran *et al.*, 2009). The extracts of *Syzygium aromaticum* were less toxic to the larvae; however their influence on development was remarkable, causing complete inhibition of adult emergence at 200 and 600 ppm concentrations of the methanol and ether extracts, respectively, against *Culex pipiens* (El Hag *et al.*, 1999).

The screened phytochemicals have insecticidal activity and their presence in *A. senegalensis* was responsible for the mortality and the inhibitory activity observed in this result. Many studies have revealed that saponins are among the most active compounds in terms of nematotoxic and insecticidal activity because of their specific interactions with the cell membranes and with the collagen proteins present on the cuticles of the larvae, causing changes in cell wall permeability and cellular death (Heng *et al.*, 2004; Argentieri *et al.*, 2008; Eguale and Giday, 2011).

Flavonoids and alkaloids are known to possess strong larvicidal properties. In fact, the activity of flavonoids is attributed to their anti-oxidative and free-radical scavenging capacity (Middleton *et al.*, 2000). Kishore *et al.* (2011) reviewed the efficacy of

phytochemicals against mosquito larvae according to their chemical nature and described the mosquito larvicidal potentiality of several plant derived secondary materials, such as, alkanes, alkenes, alkynes and simple aromatics, lactones, essential oils and fatty acids, terpenes, alkaloids, steroids, isoflavonoids, pterocarpan and lignans.

There was decrease in the life stages (number of eggs per raft, number of L1, L2, L3, L4, pupae and ratio of male to female) of *Culex quinquefasciatus* that survived exposure to ethyl acetate leaf fraction of *A. senegalensis* compared to control. The number of the life stages reduced from one stage to another progressively. There were more males than females that emerged successfully. The reduction in the life stages recorded could be as the result of phytochemical effects that caused mortality in the progeny of the survived parent. It can also be due to the effect of the plant fractions' interaction with the reproductive system of the survived mosquito which resulted in the decreased progeny survival. The general morphological appearance of the treated mosquito stages were abnormally weak compared to the control.

Duration of transformation of the life stages of *Culex quinquefasciatus* exposed to ethyl acetate fraction of *A. senegalensis* was longer than the control. The phytoconstituents of *A. senegalensis* have the ability to prolong the duration of the life stage of *Culex quinquefasciatus* as was demonstrated in this study. This study agrees with the findings of Pushpalatha (2015) who reported that treatment of *Culex quinquefasciatus* with 50% of the EC₅₀ of I instar *Culex* by *Croton hirtus* leaf extracts decreased the fecundity by 85.4%. On the other hand treatment of I instar of *Culex quinquefasciatus* with 50% of EC₅₀ *Pogostemon quadrifolius* leaf extract decreased the fecundity by 72.39% over

control. Treatment of larvae, especially with 50% of the EC₅₀ of the active fractions of the extracts significantly decreased the hatchability of the eggs oviposited by the adults obtained from them. Treatment of the larvae with 50% of the EC₅₀ of the extracts induced 86 to 91 % sterility in the progeny of *Culex quinquefasciatus*. In general, the active fraction of the two selected plant extracts decreased the fertility of the *Culex quinquefasciatus* significantly (p<0.05).

The acute toxicity of compound ergosterol on larvae of *Culex quinquefasciatus* that was isolated from the ethyl acetate leaf fraction of *A. senegalensis* caused significant mortality of *Cx. quinquefasciatus* larvae. The mortality was dose dependent. Ergosterol, a phytosterol, was found to be responsible for the mortality of larvae and pupae, inhibition of adult emergence and effect on fecundity. Phytosterols are plant sterols. They are a group of steroid alcohols, phytochemicals naturally occurring in plants. Phytosterols are applied in medicine and cosmetics and are taken as food additives to lower cholesterol. Ergosterol is a major fungal sterol and abundant in most fungal cell membranes. Nevertheless, ergosterol is also found in relatively high amounts in plants like corn, cotton seed, peanut and linseed oils. It is either absent or found in small amounts in higher plants (Mbambo *et al.*, 2012).

Ergosterol is a small percentage of the sterol mixture in plants and animals. Stigmasterol and β -sitosterol are well known phytosterols. They are similar in structure. β -sitosterol has been shown to reinforce the membrane while stigmasterol is not. β -sitosterol and its saturated form, β -sitostanol, are known to reduce the absorption of cholesterol in the

intestinal lumen. β -sitosterol was reported as an antitumor and hypoglycaemic compound (Mbambo *et al.*, 2012).

Ergosterol exists in free and combined state, especially, as ergosterol peroxide (Liu *et al.*, 2015; Dembitsky, 2015). More than 900 endo-peroxides and hydroperoxides have been isolated from natural sources, mainly as constituents of plants, and fungi, fungal endophytes; they also were found in algae, invertebrates, and other organisms. Among naturally occurring endo-peroxides and hydroperoxides represent a large group of compounds which are shown to possess antimalarial, antibacterial and cytotoxic activities (Bu *et al.*, 2014).

From the bioactivity point of view, ergosterol has great importance since it can be converted into vitamin D₂ after photolysis and thermal rearrangement and was reported as having health-promoting properties such as antioxidant, anti-inflammatory and antihyperlipidemic activities, being also involved in the activated expression of specific defense genes. Liu *et al.*, (2015) have reported the cytotoxicity and antimicrobial activity of ergosterol; it possesses weak cytotoxicity against HL-60 and BEL-7402 cell lines and moderate antimicrobial activity against the bacteria *E. aerogenes* and *P. aeruginosa* and the fungus *C. albicans*. Ergosterol powder is an irritant to skin, eyes, and the respiratory tract. Ingestion of large amounts can cause hypercalcemia, which (if prolonged) leads to calcium salt deposits in the soft tissues and, in particular, the kidneys.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

At the initial stage of the experiments phytochemical screening by standard procedures and thin layer chromatography were carried out on the crude extracts of the selected plants to determine the classes of the constituents. Carbohydrates, sterols/ triterpens, alkaloids, tannins and flavonoids were identified. Phenolic compounds were identified by their characteristic green colouration before spraying and dark spots after spraying with vanillin-sulphuric acid (TLC of sulphuric acid). Acute toxicity test was carried out with eight ethnomedicinal plants and *Annona senegalensis* leaf ethanolic extract had the highest activity as determined by a comparison of their LC₅₀ values. Hence, histopathological examination was carried out to determine the effects of *Annona senegalensis* leaf ethanolic extract on the pre-imaginal stages of *Culex quinquefasciatus*. The result was a debilitating effect characterized by apolysis of the head region, and degeneration of the midgut, hindgut and esophagus.

In this study, the acute toxicities of aqueous and ethanolic extracts of the leaves of *Annona senegalensis* were tested on *Culex quinquefasciatus* larvae. The ethanolic extract had higher activity as compared to the aqueous extract. Hence, bioassay-guided fractionation of *A. senegalensis* crude ethanolic extract was conducted by partitioning and later by column chromatography. Aqueous, n-butanol and ethyl acetate fraction were obtained after defatting with N-Hexane. The ethyl acetate fraction had the highest activity, hence, ethyl acetate fractions of *H. asper*, *H. esculentus*, *U. lobata* and *V. amygdalina* were obtained to compare. The ethyl acetate fraction of *A.*

senegalensis retained its highest activity; it was used to determine effects on survival rates and fecundity.

The Thin Layer Chromatography (TLC) of *A. senegalensis* was carried out with various spraying agents (TLC of ethyl acetate spraying agent) and solvent systems (TLC of ethyl acetate). The n-hexane/ethyl acetate, v/v 60/40, was retained. After eluting on Column, a preparative TLC was carried out on the ethyl acetate fraction of *Annona senegalensis* fraction to compare with the crude extract. Nine subfractions were identified by characteristic colourations. Subfraction 9 did not give any clear spots so it was discarded (Control of ethyl acetate of *A. senegalensis*). Subfractions that gave similar spots were pooled together into compounds (A₁ and A₂), B (B₁ and B₂) and C.

Compound B renamed “YU” was identified as a pure compound. It was colourless and dissolved in chloroform. It gave R_f value of 0.57 (chromatogram of compound “YU”). Acute toxicity of “YU” was carried and 10.00mg of the compound was sent for NMR analysis and identified as ergosterol, a sterol.

6.2 Conclusions

- i. Alkaloids, sterols/triterpenes, saponins, flavonoids, tannins, carbohydrates and glycosides were present in the plants. Anthraquinones were present only in *Annona senegalensis* and *Vernonia amygdalina*
- ii. The median lethal concentrations measured in mg/ml of the ethnobotanical plants were; *A. senegalensis* (0.457mg/ml), *H. asper* (4.032mg/ml), *H. cannabinus* (1.237mg/ml), *H. esculentus* (0.571mg/ml), *H. lunariifolius*

(2.568mg/ml), *H. sabdariffa* (2.193mg/ml), *U. lobata* (0.625mg/ml) and *V. amygdalina* (0.536mg/ml)

- iii. The ethanolic extract of *Annona senegalensis* leaf caused apolysis of the head cuticle, separation of the basement membrane from the midgut epithelium and the degeneration of the microvilli on *Culex quinquefasciatus* larvae
- iv. The ethyl acetate fraction of *Annona senegalensis*, *Hibiscus esculentus*, *Urena lobata* and *Vernonia amygdalina* inhibited adult emergence in *Culex quinquefasciatus*. The ethyl acetate fraction of *Annona senegalensis* had the highest effect. There was a reduction in the oviposition (from 101 eggs in control to 70 eggs in experimental survivors) of the emergent adults of *Culex quinquefasciatus* larvae that were exposed to ethyl acetate fraction of *A. senegalensis* leaf
- v. The active compound responsible for the mortality of larvae, inhibition of pupae and adult emergence is suspected to be ergosterol, a phytosteroid.

6.3 Recommendations

Based on the findings of the study, the following recommendations are hereby, given:

- i. The experimental plants contained phytochemicals that could be exploited to develop insecticide for mosquito control.
- ii. Further study need to carry out to assess the out effect of the leaf fraction of *A. senegalensis* on *Cx. quinquefasciatus* larvae.
- iii. Effect of ethyl acetate leaf fraction of *A. senegalensis* need to be examined on other mosquito species.

- iv. Stem and roots extracts of *A. senegalensis* need to be evaluated for the larvicidal activity.

Contribution to Knowledge

Title: Effects of some ethno-medicinal plants extracts on *Culex quinquefasciatus* (say)

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Programme: PhD. Zoology

The study has contributed to the scientific knowledge as follows:

- i. The larvicidal activity of *A. senegalensis* had the highest mean larval mortality of 23.75 (95.00%), while *H. asper* (11.50; 46.00%) had the least; similarly, *A. senegalensis* and *H.asper* had LC₅₀ of 0.457mg/ml and 4.032 mg/ml respectively.
- ii. Ethanolic extract of *A. senegalensis* caused higher mean mortality (11.18; 44.72%) of the mosquito larvae in comparism with the aqueous extract (4.44; 17.76%).
- iii. The activities of the selected solvents used in this study at concentration 10.00 mg/mlwere as follows: Ethyl acetate leaf fractionof *A. senegalensis* recorded an average mortality of 23.50 (94.00%), followed by n-hexane (13.18; 52.72%), n-butanol (5.25; 21.00%), while aqueous fraction had the least (2.25; 9.00%).
- iv. The acute toxicity of the isolated compound (ergosterol) from the leaf fraction of *A. senegalensis* on *Cx. quinquefasciatus* was dose-dependent with the highest activity at dose 1000µg/mL (80.00%), while dose 125µg/mL had 18.00%.

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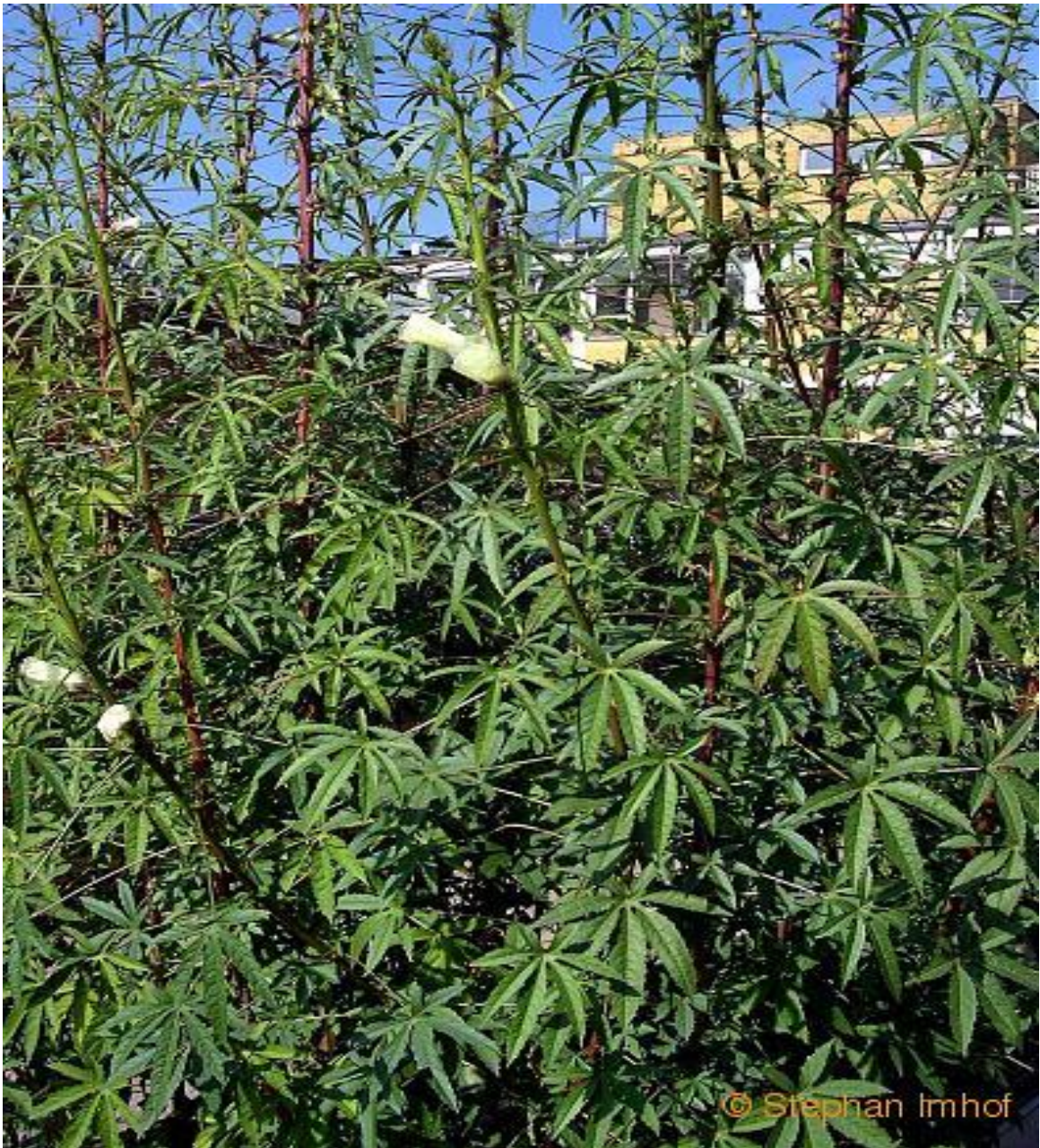
APPENDICES



Appendix I: *Annona senegalensis*



Appendix II: *Hibiscus asper*



Appendix III: *Hibiscus cannabinus*



Appendix IV: *Hibiscus esculentus*



Appendix V: *Hibiscus lunariifolius*



Appendix VI: *Hibiscus sabdariffa*



Appendix VII: *Urena lobata*



Appendix VIII: *Vernonia amygdalina*



Appendix IX: Larvicidal bioassay