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Larvicidal and phytochemical analysis of Hypoestes forskaolii extracts against Anopheles gambiae, Aedes aegypti and Culex quinquefasciatus

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LARVICIDAL AND PHYTOCHEMICAL ANALYSISOF *Hypoestes forskaolii* EXTRACTS AGAINST *Anopheles gambiae, Aedes aegypti* AND *Culex quinquefasciatus*

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

This research evaluated larvicidal potencies and phytochemical analysis of *Hypoestes forskaolii* (vahl) R. Br root extracts. Larvicidal activities were tested to *Anopheles gambiae, Aedes aegypti* and *Culex quinquefasciatus.* World Health Organization protocol for evaluating and testing insecticides was adopted with minor modification. Larvae of these mosquitoes were allowed to interact with extracts prepared in different concentration ranging between 25 to 750 µg/mL and the death rate were noted subsequent to 24 h, 36 h and 72 h. Root extracts of *H. forskaolii* displayed its effectiveness towards larva's of *A. gambiae, A. aegypti* and *C. quinquefasciatus* 3rd instars having LC_{50} between 3.8989 to 220.4789 µg/mL for *A. aegypti*, LC_{50} of 2.0322 to 69.6596 µg/mL for *A. gambiae* and LC_{50} of 6.0004 to 177.5595 µg/mL for *C. quinquefasciatus*. The chloroform extract results indicated high mortality of larvae subsequent to 72 h of contact for the three species of mosquitoes tested. *Anopheles gambiae* had the LC_{50} of 2.0322 µg/mL where by *A. aegypti* had LC50 of 3.8989 µg/mL while *C. quinquefasciatus* showed LC50 of 6.0004 µg/mL. Analysis of the organic compounds found in *Hypoestes forskaolii* chloroform extract was performed using gas chromatography-mass spectrometry technique (GC-MS). Twenty three compounds were identified namely; piperonal, caryophyllene, caryophyllene oxide, β-humulene, α-farnesene, Nerolidol, patchoulane, γ-cadinene, viridiflorol, *n*-hexadecanoic acid, octadecanoic acid, bicyclo [5.2.0] nonane, 2-methylene-4,8,8-trimthyl-4vinyl, Kaurene, 9,12-octadecadienoic acid *Z, Z,* eicosane, *allo*- aromadendrene oxide, Epiglobulol, Longipinane, curcumene, Globulol, Calamenene, α-cedrene and Copaene. The larvicidal activity of *Hypoestes forskaolii* extracts is likely due to the presence of one or some of these compounds. Further study is needed to establish the compound(s) responsible for the displayed larvicidal potencies.

DECLARATION

I, Albert Joseph Sillo do hereby declare to the senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

..... 13/08/2020

Albert Joseph Sillo **Date**

Name and signature of Candidate

The above declaration is confirmed by

..... 14/08/20

Prof. Hulda Swai **Date**

Name and signature of supervisor

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Dr. Musa Chacha **Date**

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CERTIFICATION

This is to certify that the accompanying dissertation by Albert Joseph Sillo has been accepted in Partial Fulfillment of the Requirements for the Degree of Master's in Life Sciences (Biodiversity and Ecosystem Management) of the Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania.

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Prof. Hulda Swai **Date**

Name and signature of supervisor

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Dr. Musa Chacha **Date**

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DEDICATION

This dissertation is dedicated to God for his very powerful mercies and faithfulness towards achieving this grateful work, and to my parents for their moral support during the course of study.

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LIST OF ABBREVIATIONS

CREATES	Africa Center for Excellence in Research, Agriculture advancement, Teaching Excellence and Sustainability
DDT	Dichloro Diphenyl Trichloroethane
DMSO	Dimethyl sulfoxide
FigP	Computer programme for analysis of statistical data
Fig	Figure
GC-MS	Gas chromatography-mass spectrometry technique
HCL	Highest Confidence Limit
HFCE	*Hypoestes forskaolii* chloroform root extract
HFME	*Hypoestes forskaolii* methanolic root extract
IPM	Integrated Pest Management
IMM	Integrated Mosquito Management
LC_{50}	Lethal concentration (concentration to kill 50% of organisms)
LCL	Lower Confidence limit
Mf	Molecular formula
Mwt	Molecular weight
NIST	National Institute Standard and Technology
ND	No death at all levels of concentration tested
NM-AIST	Nelson Mandela African Institution of Science and Technology
RT	Retention time in minutes
TPRI	Tropical Pesticide Research Institute
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Mosquitoes among other insects have been demonstrating their effectiveness in transmitting many tropical diseases including deadly viral disease. The total of 3000 species of mosquitoes has been identified and classified from every part of the world, but 100 species have been reported to be vectors for human diseases (Abou-Enaga, 2014). Among many groups of Arthropods mosquitoes are the most identified active species in transmitting diseases and hence it is recognized as the insect with the first interest as public enemy (Makirita *et al*., 2015; Ghosh *et al*., 2012). Mosquitoes transmit Malaria, Dengue fever, Chikungunya, Rift valley fever, Filariasis, West Nile fever and Encephalitis (Hemalatha *et al*., 2015; Dua *et al*., 2010). Around 700 million tropical inhabitants are contaminated with mosquito transmitted diseases yearly and hence social economic setbacks, poverty and death (Mavundza *et al*., 2014). Synthetic chemical pesticides are common in management of mosquitoes in the communities but improper use have established serious problems such as development of genetical mutations, high cost of pesticide application and difficulties from handling as well as environmental degradation (Adeyemi, 2010; Lee, 2000). Plants have been used in the management of destructive insects in our life history. These medicinal plants are also known to be novel; less cost and effective method of controlling vectors but the potencies for most of these plants have not been investigated in detail and become certified for scientific validation (Kilonzo *et al*., 2017). The botanical insecticides are specific to pest in control, risk free to organisms which are not in target but they are also biodegradable to the environment (Erturk *et al*., 2004; Kabaru *et al*., 2001). They are also biodegradable and harmless to the environment (Khater, 2012; Nikoletta *et al*., 2011). Conventional insecticides possesses usually one active compound but biopesticides have more than one phytochemical compounds that affects behaviors and other daily routines of the particular organisms (Parera *et al*., 2017; Rehman *et al*., 2009). Possibility for botanical pesticide to build up resistance towards insects is very low (Saxena, 1987). Various botanical extracts have also been referred to hold back the presence of these detrimental insects (Pavela, 2016; Kareru *et al*., 2013; Renault-Roger, 1997).

Ethno medicinally the plant has been used in management of malaria, amoeba, Jaundice, urine blockage, stomachache, swellings, external infections, anthrax as well as snake bite. In the treatment of amoeba the roots of *H. forskaolii* is mixed with the roots of verbanaceae boiled with milk which was then taken orally (Asnake *et al*., 2016; Jarsso, 2016; Andarge *et al*., 2015; Araya *et al*., 2015; Teklay *et al*., 2015; Kidane *et al*., 2014; Kipkore *et al*., 2014; Teklay *et al*., 2013). *Hypoestes forskaolii* was also famous in killing houseflies among pastoralist where by the barks of the root is chopped, grinded and then added to the fresh milk and exposed to the houseflies to feed on it. Though it has been widely used in management of harmful insects its larvicidal activity against any mosquito species was not yet validated. This study evaluated potencies of larvicidal activity of *H. forskaolii* root extracts in opposition to *A. gambiae, A. aegypti* and *C. quinquefasciatus* and avail the secondary metabolites responsible the activity.

1.2 Statement of the problem

Mosquitoes are among the organisms that possess multifaceted life cycle starting from aquatic life with four stages to adult that lives on dry land (Goselle *et al*., 2017). Mosquito are known to be the major group of insects that cause several tropical diseases and they are very active in diseases transmission than all other Arthropods known (Abou-Enaga, 2014). In its narrowed vein *Aedes aegypti* transmit dengue fever, yellow fever, chikungunya and zika virus (Aliyu, 2012; Kumar *et al*., 2012). *Culex quinquefasciatus* transmit lymphatic filariasis, encenphalialitis and west nile fever (Ashiwini *et al*., 2017; Ramar *et al*., 2014) while *Anopheles gambiae* commonly transmit malaria (WHO, 2003). Chemical pesticides interventions were applied for the management of harmful insects in our life history for some eras to date, but their rational use has given rise to serious problems like genetic confrontation, difficulties in handling and high cost as well as environmental degradation (Lee, 2000; Adeyemi, 2010). More efforts have been employed by the scientists all over the world to find several alternatives and one of it was to shift to the botanical insecticides which are specific to targeted pest, that has no pesticidal resistance records, biodegradable and cost affordable to the community (Khater, 2012; Nikoletta *et al*., 2011; Erturk *et al*., 2004; Kabaru *et al*., 2001). The use of plants with such records of accomplishment by many ethnic groups offers a potential solution. In Tanzania, *H. forskaolii* have been used for the management of houseflies among pastoralist communities. The concoction from the roots of this plant is mixed with milk and placed in an open area. Milk is used as insect attractants especially to

houseflies and cockroach. An insect that feeds on the product die instantly as they feed on the product. It is in this vein that the present study evaluated larvicidal activity of *H. forskaolii* against *A. gambiae, A. aegypti* and *C. quinquefasciatus*.

1.3 Rationale of the study

The study of the larvicidal activities derived from plant origin is important for the safe control of the harmful insects. This study is important since currently the control of the mosquitoes based mostly on the conventional insecticides that have a lot of negative effect to environment and the society. *Hypoestes forskaolii* has very good phytochemical compounds for the development of the product which is potential for managing mosquitoes Findings from this study will help to develop the larvicidal product from *Hypoestes forskaolii* plant for managing mosquitoes.

1.4 Objectives

1.4.1 General objective

To validate larvicidal potencies of *H. forskaolii* against *A. gambiae, A. aegypti* and *C. quinquefasciatus* and establish structures of secondary metabolites therefrom

1.4.2 Specific objectives

(i) To determine larvicidal activities of root extracts of *H. forskaolii* against *A. gambiae, A. aegypti* and *C. quinquefasciatus.*

(ii) To characterize larvicidal secondary metabolites from the chloroform root extract of *Hypoestes forskaolii* using GC-MS techniques

1.5 Significance of the study

This research revealed secondary metabolites possessing larvicidal properties from *H. forskaolii*. The identified secondary metabolites with larvicidal activity will offered the foundation for the advancement of the new classes for the larvicidal agents that are price helpful and ecologically beneficial to the society.

1.6 Research questions

(i) Which concentration from extract of *H. forskaolii* exhibit larvicidal activity against *Anopheles gambiae, Aedes aegypti* and *Culex quinquefasciatus*?

(ii) Which are the secondary metabolites present in *H. forskaolii* chloroform extract?

1.7 Delineation of the study

The present study focused on the evaluation and validation of the larvicidal potencies of *Hypoestes forskaolii* against *Anopheles gambiae, Aedes aegypti* and *Culex quinquefasciatus.* The study also focused on phytochemical composition of the chloroform root extract of *Hypoestes forskaolii* using GC-MS techniques that will lead to development of the larvicidal product for managing mosquitoes in near future.

CHAPTER TWO

LITERATURE REVIEW

2.1 General description of mosquitoes and their life cycles

Mosquitoes are vectors for most of the diseases caused by protoctist such as plasmodium as well as viral diseases like Dengue fever (Rathy *et al*., 2014). It is the fact that mosquitoes are much better known as annoyance insects than causative agents in regions with extreme cold climate. Out of 3000 species identified to at hand, 100 species are causative agents for various human diseases (Elangovan *et al*., 2008). On mitigating mosquitoes actions have only been directed to some of the very significant species either in adults or in larvae stage. Mosquitoes in nature display 4 dissimilar phases called instars which are eggs, maggots, cocoons and fully developed insect, the adult (Gutierrez *et al*., 2014). Feminine mosquitoes mate only in one occasion and generate eggs in pharses and establish succession for their next generation. Female mosquitoes can only allow mating to take place if it sucks the blood so that eggs can be fertilized and hatched (Ranasinghe *et al*., 2016). Male mosquitoes do not necessarily require sucking blood and therefore alternatively eat plant dews. Growth of eggs does not usually exceed 3 days in the tropical climates if enough food is present. The female mosquitoes look for condusive areas when they are about to lay their eggs, each after sucking blood and this behavior becomes repetitive.

Female mosquitoes lay eggs over stagnant water frequently for 3 days and they lay up to maximum of 300 eggs depending to their species. In the areas with optimum temperature such as hot climate, the eggs hatch within maximum of 3 days. Other types of species can lay their eggs on dampen line of water or over moist sludge soil and the eggs can be hatched only if inundated with water as described below (WHO, 1997).

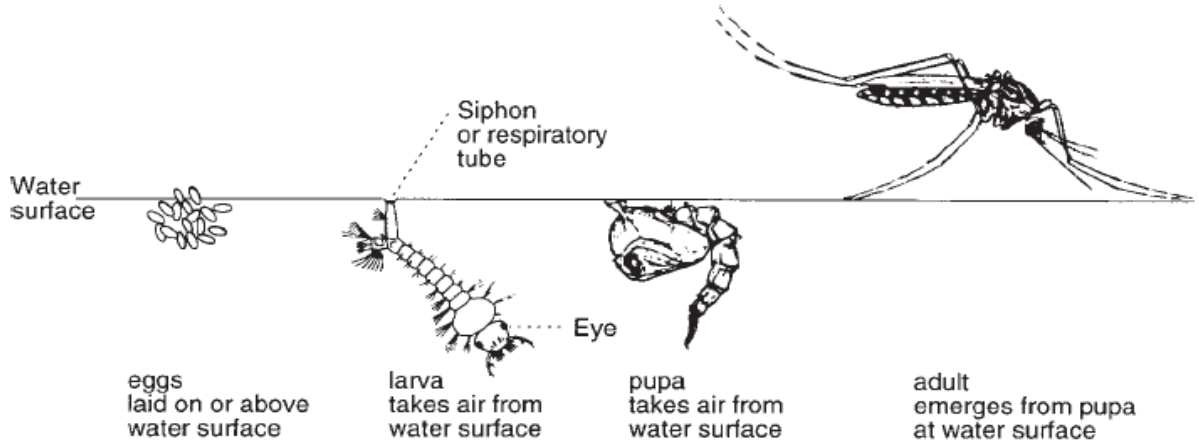

Illustration 1: Mosquito life cycle (WHO, 1997)

The eggs laid on these described wet surfaces above can remain viable for some weeks even if the surfaces become dry. When hatched these larvae grow by following all the pharses to become fully developed adult mosquito. In a favorable environment the hatching period goes up to thirteen days (WHO, 1997).

2.2 Mosquitoes eating behavior

Feeding habit in mosquitoes is governed by several factors including sex, species, habitas and feeding time. Female mosquitoes feed on humans and other animals when fertilized while male mosquitoes feed on plant dew (Merritt *et al*., 1992). Carbondioxide, heat generated by the body and odors are messengers to attract the mosquitoes to bite humans and other animals. Mosquitoes bites human beings mostly at night but biting also happens during the day. The habitats for mosquitoes range from bushes, around swamps, houses and indoors. It is very common that areas with dense vegetation are the preferred ones by mosquitoes than the open spaces (Merritt *et al*., 1992). Blood food is difficult to assimilate as a result blood fed mosquitoes look for good hidden place to allow digestion to occur.

2.3 Anopheles, Aedes and Culex mosquitoes unique features

In relation to mosquito types, 380 species of Anopheles occurs around the world and out of all 60 species are known to be typically fascinated toward humans. Culex mosquitoes also have approximately 550 species identified and recorded, and most of these are found in both hot and cold climate regions. On the other hand there are over 950 species of Aedes mosquitoes known and they are also found all over the world and they have tendency of

causing solemn biting annoyance to humans and other mammals in all climatic regions (WHO, 1997). Mosquitoes are usually divided into 2 categories which are those that suck human blood and those that do not suck blood, but they are both proficient of carrying many solemn infections. These are anophelines and culicines. Anopheles, Culex and Aedes have been considered in this study and can be distinguished from each other using different characteristics at each stage of their life (WHO, 1997). The unique feature that can easily differentiate anophelines from the rest of the mosquitoes is the size of the pulps that correlates to proboscis. Anophelines mouth parts and abdomen usually is kept in the same alignment to the surface when at rest as described in the illustration below (WHO, 1997).

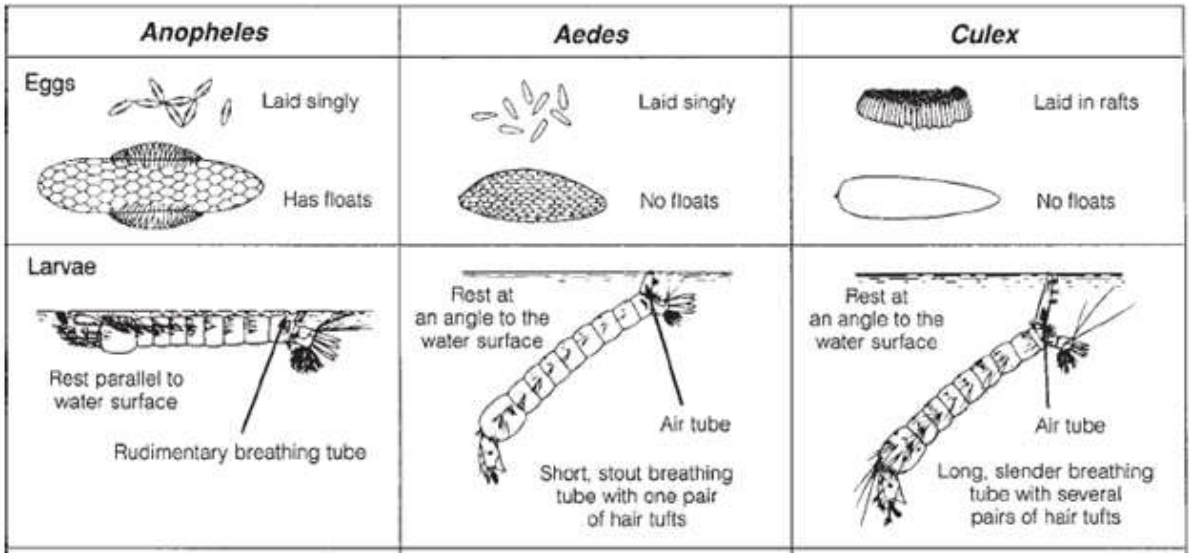

Illustration 2: Distinctive features of mosquito species at their larval stage (WHO, 1997)

2.4 Mosquitoes as the vectors for several human diseases

As it has been stated earlier mosquitoes are among of the causative agent of the serious worldwide discomfort troubles acting as carries of man diseases (Abou-Enaga, 2014). These diseases affected most of the world's population that headed to several cases of transience and morbidity with lots of economic setback (Raj *et al*., 2015). Most of the cases are reported from African continent, America and Asia (Grigoraki *et al*., 2016). The transmissions are still periodic and hence their manifestation is consolidated with numerous reasons which include type of weather change, evolution of new mosquito species, large number of tourists, immigration as well as regular visit to endemic areas (Danis *et al*., 2013). The vector borne disease outbreaks can be effectively prevented by the use of insecticides although the extreme use in both farming and communal wellbeing crooked to the materialization of defiant mosquito classes (Grigoraki *et al*., 2016).

2.5 Mosquito insecticidal resistance

Insecticidal confrontation is at serious highest peak in community wellbeing, in farming areas and in livestock keeping (Vontas *et al*., 2012). Various mosquito species are currently dead set against to all pesticide categories as the result they have accelerated this resistance year after a year (WHO, 1996). Several publications have reported defiant mosquito killings due to the evolution of these extremely resistant species (Vontas *et al*., 2012; Ranson *et al*., 2010). A good example are the Anopheles mosquito species tested from Africa that displayed high levels of insecticide confrontation in all used types of insecticides in their management mosquitoes duties (Ranson *et al*., 2011). Currently the market is rich of these insecticides with resistance while the novel one is innovated at the deliberate speed (Ghosh *et al*., 2012). It is therefore important to develop the novel and innovative botanical biopesticides for the management mosquitoes (Adeyemi, 2010).Managing insecticidal confrontation must be well thought-out and built-in the Integrated Pest Management (IPM) considered being successful management of the mosquitoes. The resistance between various groups of insecticides has to be evaluated and the immediate actions have to be implemented. Genetical factors including sudden change of the portions of the chromosomes at the point interest for the working actions of the insecticide should not be ignored (Ranson *et al*., 2010).

Understanding the strategies held responsible meant for innovative formulations and prototypes of insecticides have to be initiated by these current investigations that follows confirmed and evidenced scientific procedures. A good example is the detection and characterization of enzymes that abridgment insecticide; this will give the fundamentals of the rational design for enzyme inhibitors which modified necessary chemical compound (Vontas *et al*., 2012; Ranson *et al*., 2010).

2.6 Management of mosquitoes

Mosquitoes can be successfully controlled when harmonizing managing techniques are strategically employed in long term**.** The synthetic organochlorides, organophosphates and dichloro diphenyl trichloroethane (DDT) insecticides were comprehensively applied to overcome the transmissions of the diseases by plummeting density. Several factors including insecticidal vectors population confrontation, fumigating awareness and ecological constrains led to amendment regarding the present vector control strategies. Alternative methods were developed and built-in into the past programmes to capacitate it including the use of effective

biopesticides. The diseases transmission by vectors including mosquitoes and the idea of maintaining healthy environment were the reasons that lead to the present studies. The visions and goals for IMM are mainly focused on proper sanitation which includes removing their food, water and delineating their breeding sites for mosquitoes, water management so as to control stagnation of water, vegetation management as a protection and food for mosquito larvae and the use of larvicides and adulticidal agents. The use of biological predators and parasites should be considered in the management of mosquitoes; for example in some places bacteria and fungi have been applied in control programs. *Bacillus thuringiensis israeliensis* toxin and *Bacillus sphaericus* toxin are the best bioprospecting innovation examples of the insecticide potential in management of mosquitoes (Subramaniam *et al*., 2012). Physical barriers including restrictions through doors, air ventilation spaces and individual protections including use of gears in outdoor activities are also important. Chemical suppression or management particularly using botanical insecticides especially when the mosquitoes are in larval stage in their life cycles is highly recommended (WHO, 1996; Rose, 2001). Larviciding technique in management of mosquitoes is one of the best methods than controlling adult mosquitoes especially when the larvicides originate from botanicals. Management of mosquitoes at larval stage is also the only option in some areas where there is no any natural control to prevent the growth of mosquitoes to matured adults (Ghosh *et al*., 2011).

2.7 Management of mosquitoes using botanicals

To date over 1900 species of trees, shrubs and other kinds of vegetation are documented to possess insecticidal properties. These plants possess active chemical compounds that act as either insecticides, repellents or growth inhibitors **(**Govindarajulu *et al*., 2015). These plants are capable of controlling pests because they possess phytochemical compounds with active ingredients in it for their self defense against herbivorous of insects. Plant based pesticides is safe to ecosystem and these secondary metabolites give another option to control mosquitoes. Investigated phytochemicals are therefore good as an exchange source of insecticides in struggle towards mosquitoes **(**Govindarajulu *et al*., 2015). Among several plants investigated *Hypoestes forskaolii* have also been investigated, studied and reported in managing mosquitoes as traced from ethno medicinal information.

2.8 Taxonomical hierarchy of *H. forskaolii* plant

The genus *Hypoestes* comprises of more than 300 species, which are widely dispersed all over the areas surrounding tropical zones including Tanzania. Genus name *Hypoestes* originated from the Greek word 'hypo' that means below and 'estia', which means house .This means bracts that, cover the flowers. *H.forskaolii* is the plant species which belongs to kingdom Plantae, division Tracheophyta, class Magnoliopsida, order Lamiales, family Acanthaceae and the genus Hypoestes. The common relative includes crossandrass (firecrackers), aphelandras (zebra plants), black eyed Susans and Jusficias (shrimp plants). The plant has three subspecies which are *Hypoestes forskaolii* (vahl) R. Br, *Hypoestes forskaolii* (vahl) Roem & Schult and Asystasia mysnensis (Roth) T Anderson. *Hypoestes forskaolii* derived its name after Pehr Forsskal (1732-1763) the student of Linnaeus travelled and collected it in Egypt and the Arabian Peninsula. *H. forskaolii* possess other common names depending to the community around the world for example in Zambia it is called "*Rumanyo*". In Ethiopia, it is known as "*Dergu*". However it is commonly known as white ribbon bush in English language. In Kiswahili the plant is also known as "*Majani ya punda"* by the fact that *Hypoestes forskaolii* can only be eaten by the donkey only when it's very drought and there is no optional food for these animals.

Plate 1: *Hypoestes forskaolii* plant after seven months of planting in the field

2.9 *Hypoestes forskaolii* geographical distribution in Africa and ecology

Hypoestes is a group of dicot plants with many species which is widely dispersed all over the tropical areas and around the Indian Ocean extending to some adjacent regions including

Tanzania, Ethiopia, Kenya and Zambia, extending to Saharan highlands, Arabia and Madagascar

Figure 1: Map of Africa, the distribution of *Hypoestes forskaolii* plant (International plant names index and world checklist of selected plant families, 2018).

Hypoestes forskaolii accepted and its native range is Africa to South West Arabian peninsula. It is very widespread and often abundant species in a wide range of habitats and the most frequently encountered species of acanthaceae in tropical Africa. *Hypoestes forskaolii* was assessed as a least concern in the Red List of South African Plants (Kamundi, 2006). Ecologically *H. forskaolii* is a polymorphic species recorded from most habitats mostly common in open woodland and wooded grass land and on sand soils and rocky slopes and disturbed areas such as road sides but also occurring in river Rhine and open forest.

2.10 Ethno insecticidal and medicinal use of *Hypoestes forskaolii*

Hypoestes forskaolii has been used traditionally for the management of anthrax, malaria, amoeba, jaundice; stomach-ache, urine blockage and snake bite to human. The leaves and twigs of *H. forskaolii* are used as botanical (Kipkore *et al*., 2014). In Tanzania the plant is used as insecticidal agent against houseflies where by the barks of the root is chopped, grinded and then added to the fresh milk and exposed to the houseflies to feed on it. In treating amoeba the roots of *H. forskaolii* is mixed with the roots of verbanaceae boiled with milk and then taken orally (Teklay *et al*., 2013). Jaundice is treated by the leaves of *H. forskaolii* crushed, squeezed and the juice taken orally (Araya *et al*., 2015). *Hypoestes forskaolii* is also used in treatment of stomach ache (Kidane *et al*., 2014). Snake bite to human being is also treated by the powdered roots of *H. forskaolii* (Andarge *et al*., 2015). *Hypoestes forskaolii* leaves have also been used in urine blockage problems where the leaves

dried, crushed, added water and taken daily (Belay, 2016). Various parts of *H. forskaolii* have been used to treat anthrax by being mixed with other plants (Teklay *et al*., 2015; Teklay *et al*., 2013). Malaria is also treated by the roots of *H. forskaolii* (Asnake *et al*., 2016). Since *H. forskaolii* showed effectiveness against houseflies among pastoralist communities its efficacy can be evaluated against *A. gambiae, A. aegypti* and *C. quinquefasciatus.*

2.11 Previous biological and phytochemical investigations on *H. forskaolii*

In the study done by Musayebi *et al.* (2014) it was reported that *H. forskaolii* methanol extracts had moderate activity against *Plasmodium falciparum, Leishmania infantum, Trypanosoma cruzi* and *Trypanosoma brucei.* In the same study it was also reported that *H. forskaolii* had two dieterpenes which are hypoestenonols A and B, verticillarone and hypoestenone compounds. However *H. forskaolii* have been reported to possess cytotoxity against melanocytes (HBF4) cells of a human being (Almehdar *et al*., 2011).

2.12 *Hypoestes forskaolii* as a source of honeybees forage

Hypoestes forskaolii is one of the most important honeybee plants. The plant is important honey source and honeybees forage it for the abundant pollen and nectar. Honey bees using *H.forskaolii* produce large quantities of light and pure white honey, which has high demand and price in the market, and generate high income for the beekeepers (Haftom *et al*., 2011). However *H. forskaolii* produces white flower between Septembers to November in a year (Plate 2).

Plate 2: *Hypoestes forskaolii* plant flowering in months of September to November Source: Flora of Zimbabwe

2.13 Conservation of potential medicinal plants in general

The market thirst for remedial plants in several emergent countries gained its unsystematic harvesting of various medicinal plant species including forests. In regardless of these medicinal plants suffering consequences of dramatic decrease due to growth in agricultural sector, deforestation and infrastructure associated developments the demand in international market and lack of knowledge on conservation among traditional healers have placed a serious threat on a number of medicinal plants worldwide (Cunningham, 1996)

Despite the fact that there are organized regulated sectors for the management of sustainable use of our natural resources still medicinal plants are in high risk of being endangered or threatened due to unwise exploitation with no attention to the future. Unwise use of Medicinal plants for health support needs, earnings generation and livelihood precautions also confirm the controversy of the extinction of these important and potential resources (Hamilton, 2003; Cunningham, 1993). Massive harvest of barks, roots, and whole plants from wild populations are the major reasons to numerous extinctions.

The sustainable use of leaves, flowers, seeds and fruits should be encouraged if they contain the same bioactive chemical compounds. Conservation and cultivation of medicinal plants in botanical gardens must be given priority along with other conservation options and market incentives. Plants are important for our aesthetics as ornaments since they are beautiful and important part of our environment (Rukangira, 2001).

Kasagana *et al*. (2011) explained that the reason behind this protection is to allow biological resource to be harvested withought finishing it from the nature. This includes collection, propagation, characterization and evaluation. The protection of plant hereditary possessions has been understood to part of biodiversity conservation of the present and the future generation.

2.14 *Hypoestes forskaolii* propagation

Hypoestes forskaolii posse's alternative generation to propagate and it produces flowers which develop to the seed between September to November in a year. However in case where environment is not conducive for gametophyte generation *H. forskaolii* can be propagated by splitting, cutting, ground layering, and air layering under in-situ or under natural growing areas (Haftom *et al*., 2011). This can be best alternative of ensuring sustainable availability of

H. forskaolii in the community. *Hypoestes forskaolii* can be used as an ornament for home decoration due to its nice green color with white flowers visited by beautiful insects and its tolerance capacity to drought throughout the year.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of plants roots and preparation of extracts

Plant roots of *Hypoestes forskaolii* was taken from Endasak village in Hanang district, Manyara region. *Hypoestes forskaolii* species was classified by Dr. Epraim Njau, who is the specialist in plant identification in the National Herbarium of Tanzania and the prepared plant specimen coded was kept at NM-AIST. The plant roots were washed, chopped, blended, pulverized and sequentially macerated using chloroform and methanol for 72 h. The mixtures were exposed to rotor evaporator to obtain clear crude extracts which was then kept in refrigerator having -20°C waiting for testing time. Final residues were subjected to larvicidal activity test and to GC-MS analysis test.

Plate 3: Preparation of the chloroform and methanol root extracts of *H. forskaolii*

3.2 Preparations of mosquito's larvae

Three species of mosquito's larvae instars of 3rd stage which are *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus* were raised, identified as well as prepared at Tropical Pesticide Research Institute (TPRI) Arusha, Tanzania.

3.3 Larvicidal activity

WHO protocol of the year 1996 was adopted for larvicidal activity assay with minor modifications. Larvae of *A. aegypti* and *C. quinquefasciatus* were fed using dog biscuits while *A. gambiae* were fed with tetramine during experiments. Stock solutions for methanol

and chloroform *H. forskaolii* roots crude extracts were prepared and used in this study. 500 mg/mL of crude extracts was dissolved in 5ml of DMSO respectively. Using serial dilution concentrations in a range of 25, 50, 100, 200 and 750 µg/mL were prepared from stock solution. Each concentration was made to 100 ml by adding distilled water in disposable cups. Ten late third instars larva's of mosquito were put into the solution to test and the number of death were identified and recorded after 24 h, 36 h and 72 h. Small beakers with ten larvae of mosquitoes, purified water and 0.5 µg/ml of DMSO were considered to be control conduct test. Experiments were then conducted using four replicates in the regulated temperature of 25 ± 2 °C with humidity ranging between 75 to 85%. Dead larvas were then recognized with no capacity to become mobile and we're not able to reach water surface. The mean percentage mortality was calculated and using statistical tool the lethal concentrations (LC_{50}) required to kill the larvae of mosquitoes tested was obtained.

3.4 GC-MS analysis

GC-MS with the capacity of 6890N GC connected to 5975 MS which are the USA technologies having HP-5 column with 30 m length, 0.25 mm dimensions and 0.25 µm film thicknesses were used to analyzed phytochemical compounds. Injection volume with 1µL and the constant flowing carrier gas known as Helium in 99.999% were considered. Temprature in the injector was stabilized to around 250°C while in the ion-source the heat was 280°C but in oven heat was maintained to constant of 110°C (isothermal for 2 min), having amplification of 10°C/min to 200°C, and then from 5°C/min to 280°C, finishing with a 9 min isothermal at 280°C. Electron ionization mode with oomphs of 70 eV with the ion source heat of 230°C was operated using mass spectrometer. Inlet line heat with 200°C in GC-MS was run in 36 min. Mass spectrometry interpretations from GC-MS was conducted referring to National Institute Standard and Technology (NIST) database having over 62 000 patterns. Spectrums of detected compounds in *H. forskaolii* chloroform root extract were assimilated and compared to spectrums in NIST library. The name, molecular weight and structure of the phytochemicals in *H. forskaolii* chloroform root extract were then analsed.

Plate 4: GC-MS analysis techniques

3.5 Statistical Analysis

FigP software (Biosoft, Cambridge, UK) was used for analysis and mean percentage mortality was plotted against logarithms of concentrations. For regression equations, LC_{50}, Confidence Interval and Regression Coefficients were calculated.

CHAPTER FOUR

RESULTS AND DISCUSIONS

4.1 Results

4.1.1 Larvicidal activity results

Present study evaluated the larvicidal activities to the early 3rd instars of *A. aegypti*, *A. gambiae* as well as *C. quinquefasciatus* using chloroform as well as methanolic root extracts of *Hypoestes forskaolii* as presented in Table 1, 2 and 3 respectively. Larva's of mosquitoes were exposed to extracts prepared in dimethyl sulphoxide at deliberation series between 25 to 750 µg/mL and mortality were recorded subsequently to 24 h, 36 h and 72 h of exposure. Referring from (Dias *et al*., 2015) as well as (Komalamisra *et al*., 2005), larvicidal properties of the plant extract is measured as inactive if LC_{50} is greater than 750 µg/mL, weakly effective if the LC_{50} range from 200-750 µg/mL, moderate if LC_{50} range from 100-200 µg/mL, effective if the LC_{50} is between 50-100 µg/mL and highly effective if the LC_{50} is less than 50 µg/mL.

Results from this study displayed larvicidal activities from *H.forskaolii* against three species of mosquitoes tested, giving LC_{50} values between 220.4789-3.8989 µg/mL for *A. aegypti*, LC_{50} of 69.6596-2.0322 µg/mL for *A. gambiae* and LC_{50} of 177.5595-6.0004 µg/mL for *C. quinquefasciatus.* Chloroform and methanolic were highly effective after 72h of exposure, and this proved the extracts to be remarkably significant in controlling the larva's of mosquitoes tested. The activities were specific to particular species and this evidently discovered that chloroform extract have privileged larvicidal activity with LC_{50} of 3.8989 µg/mL against *A. aegypti* (Table 1), LC_{50} of 2.0322 µg/mL against *A. gambiae* (Table 2) and $LC_{50}$of 6.004 µg/mL against *C. quinquefasciatus* (Table 3) in 72 h of exposure. The methanolic extract had the LC50 of 11.5432 µg/mL against A. *aegypti*, LC_{50} of 9.5728 µg/mL against *A. gambiae* and LC_{50} of 6.4358 µg/mL against *C. quinquefasciatus* in 72 h of exposure. The results also showed effective, moderate and weakly effective larvicidal activity for both chloroform and methanolic extract after 24 h of contact. Chloroform extracts had LC_{50} of 154.6019 µg/mL against *A. aegypti*, LC_{50} of 177.5595µg/mL against *A. gambiae* and LC_{50} of 69.6596µg/mL against *C. quinquefasciatus*. Larvicidal effects values described by *H. forskaolii* root extract were all less than 750 µg/mL which justify its use in managing mosquito larvae tested.

Table 1: Larvicide activities of *Hypoestes forskaolii* root extracts in opposition to *Aedes aegypti*

Extract code	Time	LC_{50}(µg/mL)	95% (UCL-LCL)	R^2	Regression equation
HFCE	24h	154.6019	1706.3408-14.0076	0.938	y=10.57logx +26.86
	36h	15.0053	110.7175-2.0336	0.93	y=10.44logx +37.72
	72h	3.8989	22.1742-0.6856	0.87	y=12.15logx + 42.82
HFME	24h	220.4789	904.4034-53.7492	0.994	y=18.77logx +6.015
	36h	56.3484	226.2731-14.0323	0.946	y=17.02logx +20.20
	72h	11.5432	54.3812-2.4502	0.96	y=14.28logx +34.83
CONTROL	NM	-	-	-	-

KEY:

HFCE - *H. forskaolii* chloroform root extract, HFME - *H. forskaolii* methanolic root extract, ND- No death in each concentration tested .HCL-Highest Confidentiality Limit, LCL-Least Confidentiality limit, LC_{50} -Lethal Concentration, Confidentiality Interval and R^2-Regression Coefficient

Table 2: Larvicide activities of *Hypoestes forskaolii* root extracts in opposition to *Anopheles gambiae*

Extract code	Time	LC_{50}(µg/mL)	95% (UCL-LCL)	R^2	Regression equation
HFCE	24h	69.6596	330.1227-14.6989	0.853	y=15.03logx + 22.30
	36h	8.8111	33.1905-2.3391	0.954	y=16.19logx + 34.70
	72h	2.0322	6.6260-0.6233	0.866	y=16.82logx +44.82
HFME	24h	37.1001	159.9947-8.6029	0.984	y=16.00logx +24.89
	36h	7.4977	25.1042-2.2393	0.940	y=17.43logx +34.75
	72h	9.5728	15.22-1.5400	0.973	y=8.965logx +68.02
CONTROL	NM	-	-	-	-

KEY:

HFCE - *H. forskaolii* chloroform root extract, HFME - *H. forskaolii* methanolic root extract, ND- No death in each concentration tested. HCL-Highest Confidentiality Limit, LCL-Least Confidentiality limit, LC_{50} -Lethal Concentration, Confidentiality Interval and R^2-Regression Coefficient

Table 3: Larvicide activities of *H. forskaolii* root extracts in opposition to *Culex quinquefasciatus*

Extract code	Time	LC50(µg/mL)	95% (UCL-LCL)	R^2	Regression equation
HFCE	24h	177.5595	1661.1320-18.9794	0.856	y=11.43logx + 24.29
	36h	18.0962	97.3554-3.3637	0.933	y=13.51logx +33.01
	72h	6.0004	27.4143-1.3133	0.851	y=13.93logx +39.16
HFME	24h	137.7328	530.6684-35.7471	0.96	y=18.70logx +10.55
	36h	31.7442	112.8697-8.9271	0.97	y=18.02logx +22.94
	72h	6.4358	23.0496-1.7961	0.972	y=16.51logx +36.65
CONTROL	NM	-	-	-	-

KEY:

HFCE - *H. forskaolii* chloroform root extract, HFME - *H. forskaolii* methanolic root extract, ND- No death in each concentration tested. HCL-Highest Confidentiality Limit, LCL-Least Confidentiality limit, LC_{50} -Lethal Concentration, Confidentiality Interval and R^2-Regression Coefficient

4.1.2 GC-MS results

GC-MS systems were considered to spot out twenty three compounds present in *Hypoestes forskaolii* root extracts prepared by using chloroform. Retention time, peak areas, molecular weights, molecular formulas as well as bioactivity of the phytochemical compounds were presented in Table 4. The phytochemical compounds obtained feel right to metabolites classes known as sesquiterpenes, dieterpenes, fatty acids and alkane. Sesquiterpenes composed large extent than the other secondary metabolites found in *H. forskaolii* chloroform root extract. The sesquiterpenes identified were Piperonal, Caryophyllene, Caryophyllene oxide , β-Humulene, α-farnesene, Nerolidol, Patchoulane, γ-Cadinene, Viridiflorol, Bicyclo [5.2.0] nonane, 2-methylene-4, 8, 8-trimthyl-4vinyl, Alloaromadendrene oxide , Epiglobulol, Longipinane, Curcumene, Globulol, Calamenene, α -Cedrene and Copaene (Fig. 1). The fatty acids identified were *n*-hexadecanoic acid, 9, 12, -Octadecadienoic acid *Z,* Z and Octadecanoic acid while diterprene Kaurene as well as Eicosane alkane were also identified (Fig. 2).

4.2 Discussion

To easily and successfully manage mosquitoes it is better to consider their life cycle that starting from larvae stage. Larvae are easily targeted in their breeding sites which are

stagnant water that can be easily accessed, but the use of artificially formulated pesticides are harmful to human being as well as ecosystem (Abagavan *et al*., 2011; Subramiam *et al*., 2012). Biopesticides resulting from plants are therefore shows potential means especially for managing these larvae of mosquitoes. *Hypoestes forskaolii* has been ethinomedicaly used to cure mosquitoes borne ailments in Africa (Asnake *et al*., 2016; Uzair *et al*., 2015; Hemalatha *et al*., 2015; Warikoo *et al*., 2012). Currently Musayeib and Cowokers (2014) reported antiprotozoal activity of methanolic extracts against *Plasmodium falciparum, Leishmania infantum*, *Trypanosoma cruzi* and *Trypanosoma brucei.*

The present study evaluated the larvicidal activity of *H. forskaolii* against *A. aegypti*, *A. gambiae* and *C. quinquefasciatus* and generally the mortality occurred up to 50% with LC_{50} between 220.4789 and 2.0322 in chloroform and methanol extracts. The activity indicates that it was concentration dependant as demonstrated by the variations in concentration as time increases. In all experiments done the LC_{50} values obtained were not greater than750 µg/mL and hence all the LC_{50} values are potential for developing the botanical larvicidal agent for managing mosquitoes in various concentrations with range of choice. Considering the efficacy of *H. forskaolii* root extracts against *A. aegypti* the LC_{50} values ranged from 220.4789 to 3.8989 which were moderately effective to highly effective in the classification of the effectiveness of the larvicides as stated in the results section with the LC_{50} values ranging from 200 to 50 µg/mL. After 72 h the LC_{50} values range from 11.5432 to 3.8989 which is also highly effective by the fact that LC_{50} values are classified that the values are less than 50 µg/mL as referred in Fig. 2 below:

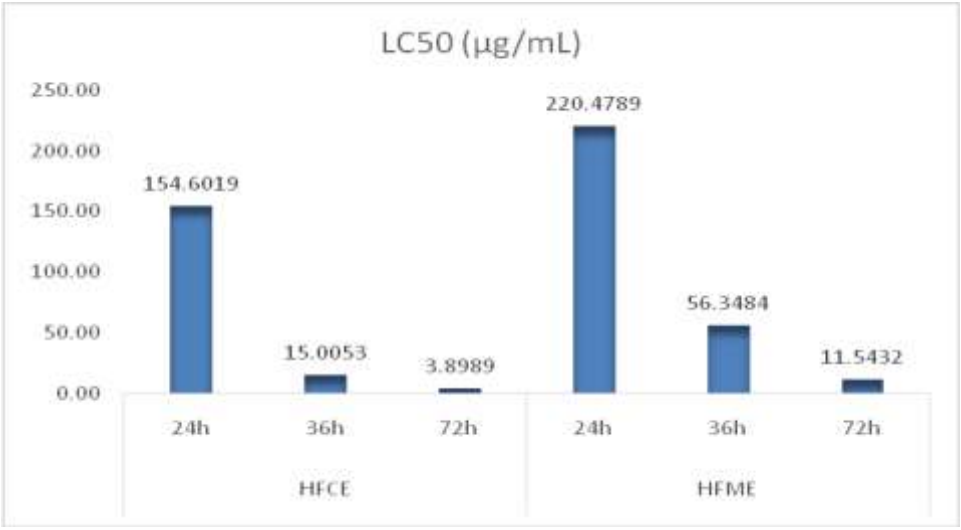

Figure 2: Larvicide activities of *Hypoestes forskaolii* root extracts in opposition to *Aedes aegypti*

Larvicides activity from *Hypoestes forskaolii* root extracts towards *Anopheles gambiae* also demonstrated active larvicidal activity where the LC_{50} values were ranging from 69.6596 to 2.0322 µg/mL which were classified as effective to highly effective lethal concentrations. After 72 h of exposure the most highly effective extracts was chloroform extract with LC_{50} 2.0322 µg/mL and followed by methanolic extract with LC_{50} value of 9.5728 µg/mL. Although LC_{50} values recordings after 24 h of exposure for both chloroform and methanol extracts are also promising for the formulation of larvicidal product for the management of *Anopheles gambiae* species as shown in Fig. 3 below:

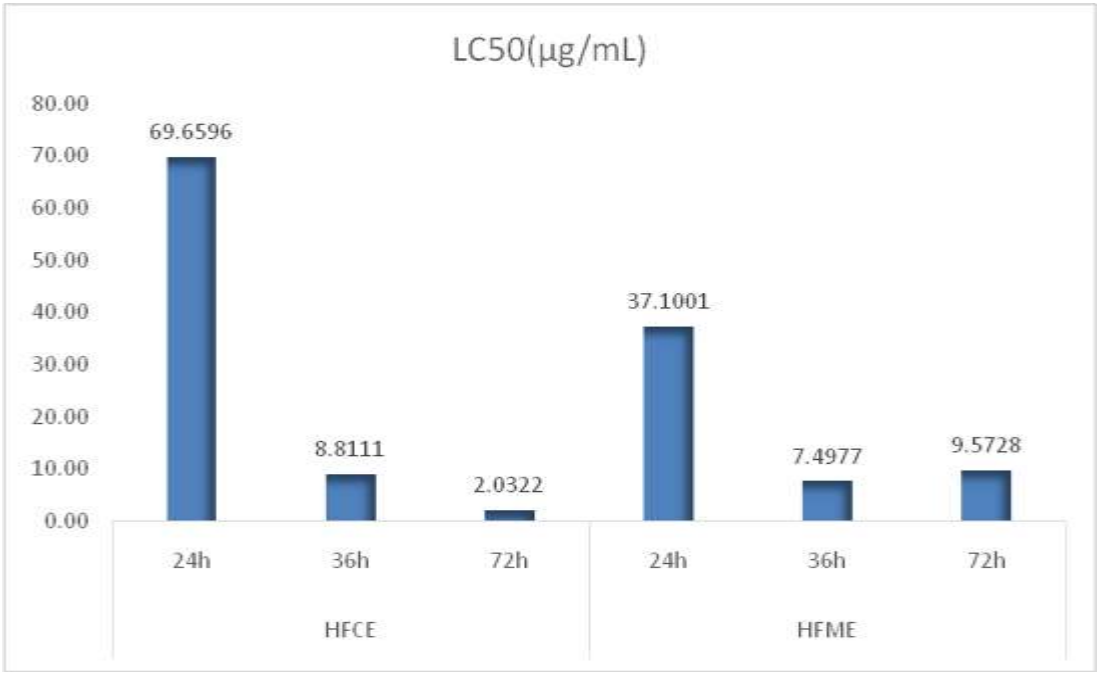

Figure 3: Larvicidal activity of *Hypoestes forskaolii* root extracts against *Anopheles gambiae*

In *Culex quinquefasciatus* species the level of resistance to *H. forskaolii* chloroform and methanol root extracts ranged within the LC_{50} values from 177.5595 to 6.0004 µg/mL. These values are still suitable for establishing grounds for developing safety larvicidal product for controlling *C. quinquefasciatus* species. The LC_{50} values ranged between moderate to highly effective concentration levels which are also suitable for sustainable control of mosquito species. After 72 h of exposure the LC_{50} values were 6.4358 µg/mL and 6.0004 µg/mL which were classified as highly effective but after 24 h lethal concentrations was 177.5595 as well as 137.7328 µg/mL. The result for chloroform and methanol extracts therefore indicates that *C. quinquefasciatus* mosquitoes can be managed by *H. forskaolii* extracts (Fig. 4)

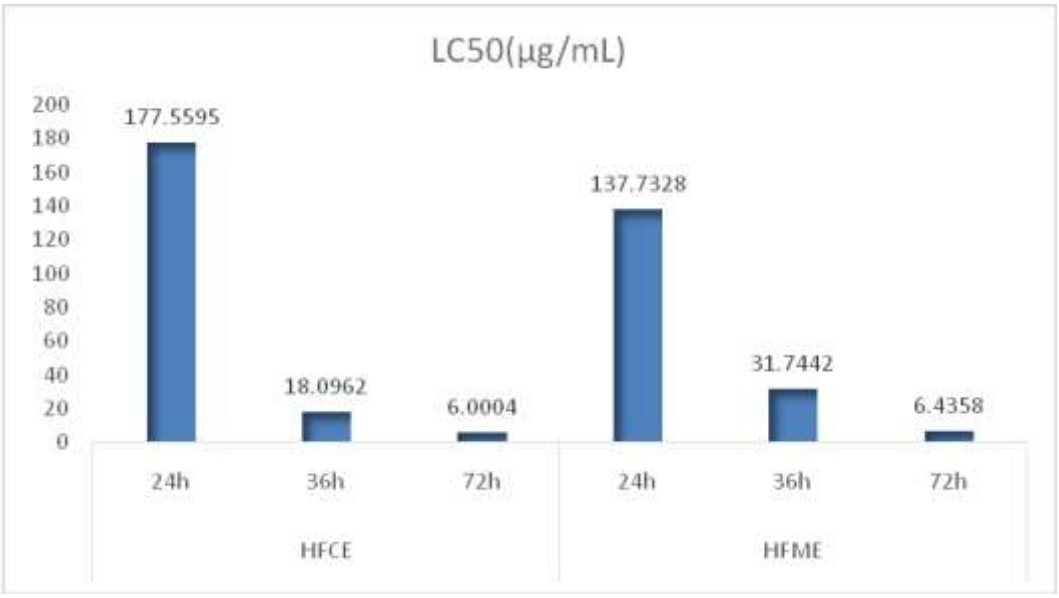

Figure 4: Larvicidal activity of *Hypoestes forskaolii* root extracts against *Culex quinquefasciatus*

The results from *Hypoestes forskaolii* root extracts therefore justify its use from ethinomedicinal information. Botanical insecticides are given priorities due to minor toxicity levels and safeness of the ecosystem than conventional pesticides (Jayapriya *et al*., 2015). Results of the present investigations exposed the fact realizing the extracts of *H.forskaolii* possessed remarkable larvicidal activity against *A. aegypti*, *A. gambiae* and *C. quinquefasciatus.* Therefore development of the larvicidal product from the *H. forskaolii* root extract as a botanical source of pesticide should be put in action because it has been investigated and showed the remarkable and promising array of phytochemical compounds present for larvicidal activity.

The GC-MS technique were considered to investigate *H. forskaolii* chloroform root extracts (Kilonzo *et al*., 2017). Secondary metabolite classified to sesquiterpenes, dieterpenes and fatty acids was recognized. The phytochemical compounds identified have been reported to demonstrate exciting bioactivities that work for cure of various ailments as reported in Table 4.

Antiinflamatory activities were recorded to be exhibited by phytochemical compounds which are 9, 12, -octadecadienoic acid *Z, Z*, caryophyllene, Bicyclo [5.2.0] nonane, 2-mthylene-4, 8, 8-trimthyl-4-vinyl, Octadecanoic acid and Kaurene (Kilonzo *et al*., 2017; Cheng-fang *et al*., 2015; Arunkumark, 2013; Leandro *et al*., 2012). However Bicyclo [5.2.0] nonane, 2-

methylene-4, 8, 8-trimethyl-4-vinyl- the phytochemical compound known to possess not only antiinflammatory activities but also antihyperlipidemic properties (Prakasia *et al*., 2015).

Phytochemical compounds that have been recorded to display larvicidal/insecticidal properties includes caryophyllene oxide, *n*-hexadecanoic acid, α-farnesene, caryophyllene, β-humulene and Patchoulane (Kilonzo *et al*., 2017; Prabodh *et al*., 2014; Zekeya *et al*., 2014; Alejandro *et al*., 2013; Murugesan *et al*., 2012; Rajeswari *et al*., 2011). Curcumene, *n*-hexadecanoic acid and γ-Cadinene compounds also demonstrated to have antioxidant activity (Otitolaiye *et al*., 2016; Aditi *et al*., 2013; Meenakshi, 2013). Caryophyllene oxide compound has also been reported to function as trypanocidal, antiedemic, antifeedant, antiinflammatory and antitumor activities (Polanco-Hernández *et al*., 2013).

Phytochemical compounds with the purpose of exhibiting antimicrobial activity were Globulol, Kaurene, *Allo*aromadendrene oxide, α-Cedrene, Copaene and viridiflorol (Sahi, 2016; Murugesan *et al*., 2012; Leandro *et al*., 2012; Sousa *et al*., 2012; Jain *et al*., 2012; Solis *et al*., 2004). A number of pharmaceutical researches have been performed with Kaurene compound in determination anti-inflammatory, bactericidal and toxicity effects. The possible toxicity from kaurenoic acid tested to sea urchins and inhibitory activities for tumor cells growth were also studied (Leandro *et al*., 2012). Therefore identifying kaurenoic acid in *H. forskaolii* root extract is not only important to larvicidal agent formulation for mosquito management but also to the other pharmacological bioactivities.

Alloaromadendrene oxide, Nerolidol, Curcumene, α–Cedrene and Viridiflorol have been reported to have antifungal activity (Sahi, 2016; Curvelo *et al*., 2014; Murugesan *et al*., 2012). Siqueira *et al*. (2001) also studied essential oil from leaves of *D. glabriuscula* pinpointed that *Allo*aromadendrene showed toxicity on *A. salina* with LC_{50} 1.6 g/mL. Regardless of 0.1% concentrations in oils of *D. lanceolata*, *Allo*aromadendrene demonstrated toxicity with LC_{50} value 7.8 µg/mL as the active substance responsible compound for that activity.

Sivasubramanian *et al*. (2013) also studied on cytotoxicity potentials of the ethanol extract of aerial parts of an Asteraceae drug source *Centratherum punctatum* cass and reported that the plant has been used to control various ailments like cancer, inflammation, intestinal disorders, fever and pain. The same authors also reported that compounds Viridiflorol, Hexadecanoic acid and Eicosane possess anticancer activity.

Table 4: Reported biological activities of volatile phytochemical compounds detected in *H. forskaolii* chloroform root extract

SN	RT (min)	Area (%)	Name	M/f	M/Wt	Bioactivity	References
1	5.820	0.14	Piperonal	$C_8H_6O_3$	150	Repellent	Dambolena *et al*. (2016) and Chen *et al*. (2017)
2	24.313	0.20	Caryophyllene oxide	$C_{15}H_{24}O$	220	Larvicidal, Insecticidal	Magalhaes *et al*. (2010) and Rajeswari *et al*. (2011)
3	17.063	0.31	Nerolidol	$C_{15}H_{26}O$	222	Antifungal	Curvelo *et al*. (2014)
4	19.306	1.05	9, 12 - octadecadienoic acid (*Z, Z*)	$C_{18}H_{32}O_2$	208	Insecticidal, Nematicide	Zekeya *et al*. (2014), Jananie *et al*. (2011) and Isijola *et al*. (2018)
5	15.844	0.89	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	256	Antioxidant, Larvicidal	Meenakshi.(2013) and Romeh, (2013)
6	17.515	0.51	α –Farnesene	$C_{15}H_{24}$	204	Insecticidal	Kilonzo *et al*. (2017) and Zhang *et al*. (2010)
7	18.374	0.43	Caryophyllene	$C_{15}H_{24}$	204	Anti-inflammatory, Antitumor	Cheng-fang *et al*. (2015) and Damasceno *et al*. (2017)
8	21.658	1.02	β- Humulene	$C_{15}H_{24}$	204	Insecticidal	Satyal *et al*. (2014)
9	20.285	0.65	Bicyclo [5.2.0] nonane, 4 - Methylene-2, 8, 8-Trimethyl-2-vinyl	$C_{15}H_{24}$	204	Anti-inflammatory	Prakasia *et al*. (2015)
10	19.741	0.28	Octadecanoic acid	$C_8H_{12}O$	124	Nematicide, Anti-inflammatory	Amala *et al*. (2014), Kilonzo *et al*. (2017)
11	18.511	0.62	Kaurene	$C_{20}H_{32}$	272	Antibacterial, Anti-inflammatory	Leandro *et al*. (2012)
12	31.866	0.12	Eicosane	$C_{20}H_{42}$	282	Antitumor	Karanja *et al*. (2012) and Nandhini 2015
13	12.119	0.26	Alloaromadendrene	$C_{15}H_{24}O$	220	Antibacterial, Antifungal	Sahi. (2016) and Chen *et al*. 2017
14	11.124	0.20	Epiglobulol	$C_{15}H_{26}O$	222	Antiseptic, Antioxidant, Anti inflammatory	Jain *et al*. (2012) and Mohammed *et al*. (2016)
15	10.592	0.13	Longipinane	$C_{15}H_{26}$	206	Anti-pedant	Otitolaiye *et al*. (2016)
16	7.158	0.07	Curcumene	$C_{15}H_{22}$	202	Antifungal, Antioxidant	Otitolaiye *et al*. (2016)
17	6.535	0.09	α –Cedrene	$C_{15}H_{24}$	204	Antifungal, Antibacterial	Murugesan *et al*. (2012)
18	13.321	0.21	Globulol	$C_{15}H_{26}O$	222	Antibacterial	Sahi. (2016)
19	8.206	0.14	Calamenene	$C_{15}H_{22}$	202	Antioxidant ,Antimicrobial	Azevedo *et al*. (2013)
20	7.982	0.06	γ - Cadinene	$C_{15}H_{24}$	204	Insecticidal, Antioxidant	Murugesan *et al*. (2012) and Aditi *et al*. (2013)
21	12.514	0.09	Viridiflorol	$C_{15}H_{26}O$	222	Anticancer, Antibacterial, Antifungal	Sivasubramanian *et al*. (2013) and Jain. (2012)
22	25.806	0.68	Patchoulane	$C_{15}H_{26}$	206	Insecticidal	Alejandro *et al*. (2013)
23	7.982	0.06	Copaene	$C_{15}H_{24}$	204	Antibacterial	Solis *et al*. (2004)

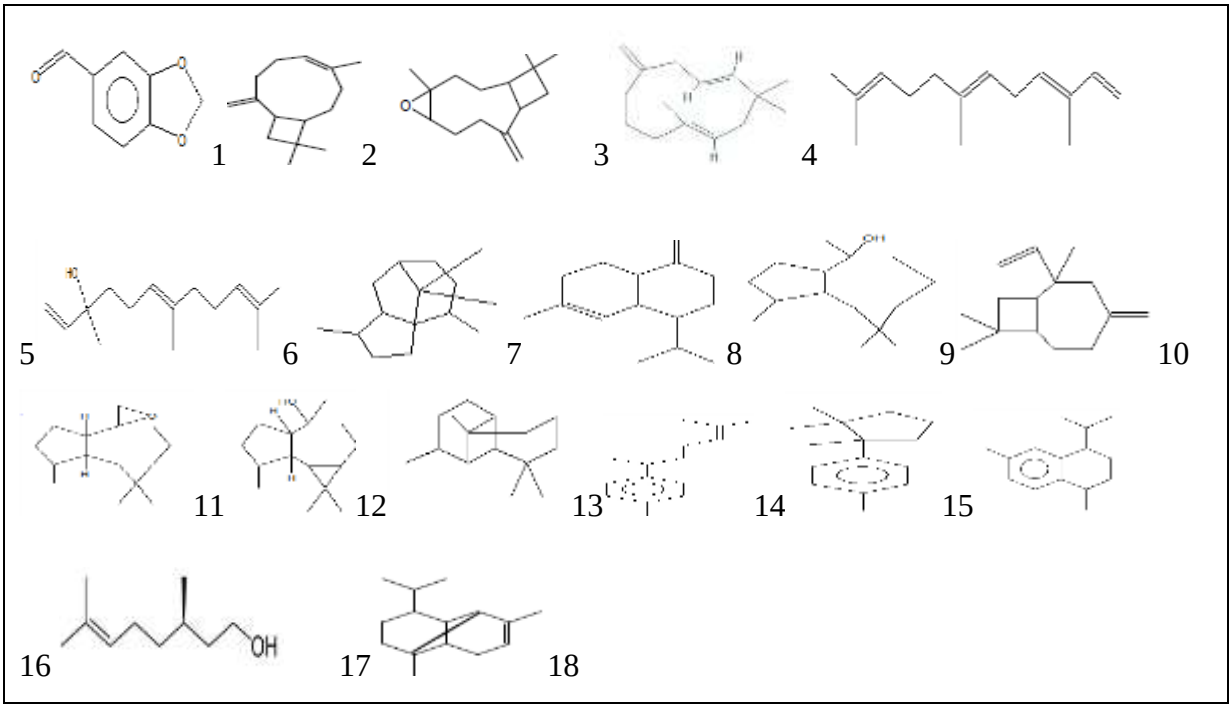

Figure 5: Structures of Piperonal (1), Caryophyllene (2), Caryophyllene oxide (3), β-Humulene (4), α-farnesene (5), Nerolidol (6), Patchoulane (7), γ-Cadinene (8), Viridiflorol (9), Bicyclo [5.2.0] nonane, 2-methylene-4, 8, 8-trimthyl-4vinyl (10), Alloaromadendrene oxide (11), Epiglobulol (12), Longipinane (13), Curcumene (14), Cuparene (15), Calamenene (16), α -Cedrene (17) and Copaene (18) identified from *H. forskaolii* chloroform root extract

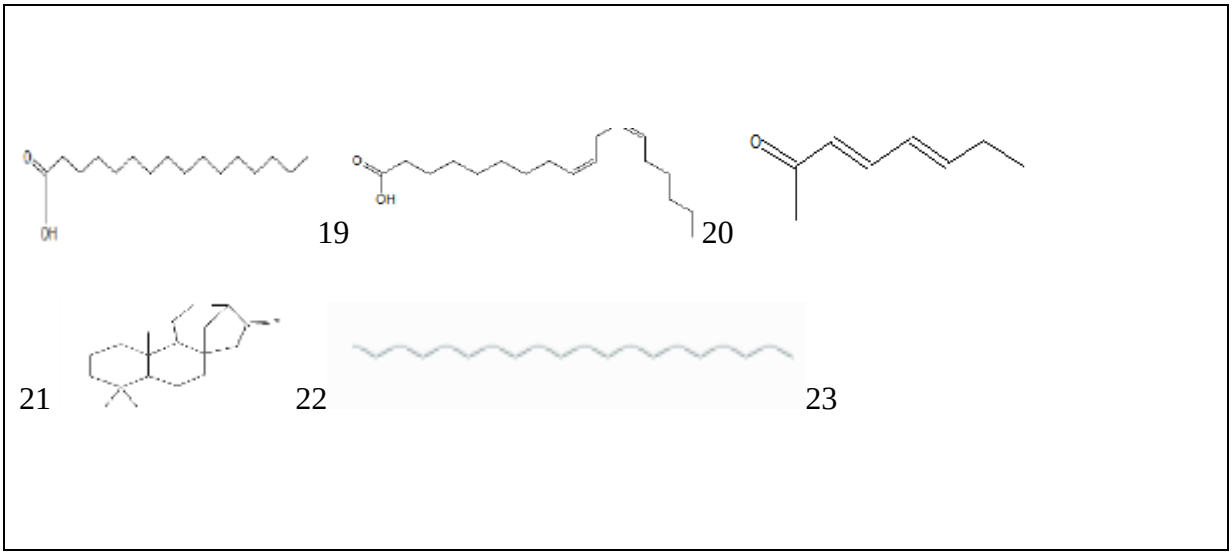

Figure 6: Structures of n-hexadecanoic acid (19), 9, 12, -Octadecadienoic acid Z, Z (20), Octadecanoic acid (21), Kaurene (22) and Eicosane (23) identified from *H. forskaolii* chloroform root extract

Caryophyllene and Eicosane have also been documented to have antitumor potencies (Rency *et al*., 2015; Sivasubramanian *et al*., 2013) while the compound Viridiflorol posse's anticancer activity (Sivasubramanian *et al*., 2013). The compound Epiglobulol have been reported to be antiseptic (Jain *et al*.,2012) while Longipinane compound have been reported to be antipedant (Otitolaiye *et al*., 2016), *n*-hexadecanoic and Octadecanoic acid have been reported to have Nematicide activity (Rency *et al*., 2015; Amala *et al*., 2014; Archana *et al*.,2014; Meenakish *et al*.,2013; Romeh,2013; Jananie *et al*.,2011).

Amala *et al*., (2014) also depicted that octadecanoic acid which is the fatty acid compound was present in the methanolic extracts *Terminalia chebula* and *Terminalia bellirica* and been used for hypercholesterolemic, antiarthiritic, antiinflammatory, antimicrobial, hepatoprotective and Nematicide. Caryophyllene oxide as one among the chemical compounds reported has been investigated for larvicide activities against *A. aegyptiae* (Magalhaes *et al*., 2010).

Sousa *et al*. (2012) in a nut shell reported the fact for caryophyllene oxide which was identified in *Solanum erianthum* and *Solanum macranthum.* Essential oils from these plants inhibited development of microbes while caryophyllene oxide demonstrated antifungal activity against *Candida albicans.* Bioactivities obtained from this investigation can be used as bench mark in using of *H. forskaolii* managing variety of diseases. Therefore plants including *H. forskaolii* are potential sources for larvicidal agents that can be used in development of the potential larvicidal product in managing larva of *A. gambiae, A. aegypti* and *C. quinquefasciatus*

CHAPTER FIVE

CONLUSION AND RECOMMENDATIONS

5.1 Conclusion

Hypoestes forskaolii plant root extracts showed very effective larvicidal activity against *A. gambiae, A. aegypti*, *C. quinquefasciatus Say*; therefore it can be used in formulation of potential product for the management of mosquitoes. The GC-MS analysis of *H. forskaolii* chloroform root extract led to identification of low molecular weight phytochemicals which verified the use of *H. forskaolii* in controlling mosquitoes. These phytochemicals are grouped as sesquiterpenes, dieterpenes, fatty acids and alkane. Findings from this study have therefore validated the medicinal potential of *H. forskaolii* chloroform and methanol root extracts including formulation of the larvicidal products.

Botanical larvicides can be processed in various ways, principally as crude plant material in the form of powder or dust. It can be as extracts from plant resins formulated into liquid concentrations. Pure isolated constituents by extraction, chromatographic techniques or hydro distillation of the plant tissues can be the best of processing pesticide.

Technically the lower the values the more effective the product will be (Okwute, 2012). The LC_{50} which is the concentration required to be killing up to 50% of the *A. gambiae, A. aegypti* and *C. quinquefasciatus* have been demonstrated in this study with promising results for both chloroform and methanol extracts. Therefore these information for *H. forskaolii* can be used to formulate the larvicidal agent and useful in managing of these important vectors of human diseases.

5.2 Recommendations

Hypoestes forskaolii plant can be conserved and managed by encouraging their growth in special places, due to the fact that it can be also grown artificially by using its stems. *Hypoestes forskaolii* can be grown in a range of climate as it also withstands harsh environment like in salt areas, drought areas and rocky land and along the roads. Considering its greenish colour throughout the year and beauty white flowers *H. forskaolii* can also be planted as an ornament in various respective places such as churches, mosques, offices, schools, offices, higher learning institutions and research institutions.

Since *H. forskaolii* is also potential for production of quality white honey it can also be planted in various open areas such as grave yards, scared grooves, farm margins, river banks, road sides, live fences of gardens and fields. Asfaw (2001) also documented that medicinal plants can be conserved by using appropriate conservational methods in gene banks and botanical gardens. These types of conservation of medicinal plants can also be possible in home gardens, as the home garden is deliberate and ideal farming system for the conservation, it can also be employed for the production which can be used to increase marginalized income by selling to the pharmaceutical industries by the community.

REFERENCES

Abou-Enaga, Z. S. (2014). Insecticidal bioactivity of ecofrinedly plant origin chemicals against *Culex pipiens* and *Aedes aegypti* (Diptera: Culicidae), *Journal of Entomology and Zoology Studies*, *2*(6), 340-347.

Adeyemi, M. M. H. (2010). Potential of secondary metabolites in plant material as detergents against insect pests: A review. *African Journal of Pure and Applied Chemistry, 4*(11), 243-246.

Ahsan, T., Chen, J., Zhan, X., Irfan, U., & Wu, Y. (2017). Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by *Streptomyces* strain KX852460 for the biological control of *Rhizoctonia solani* AG‑3 strain KX852461 to control target spot disease in tobacco leaf. *Springer Open Journal AMB Express*, *7*(54), 2-9

Aliyu, A. (2012). Vector Control Using Insecticides, Insecticides Pest Engineering, *Dr. Farzana Perveen Education,* ISBN: 978-953-307-895-3:http://www.intechopen. com/books/insecticides-pestengineering/vector-control-using insecticides. Accessed on 2018/07/26.

Almehdar, H., Abdallah, H. M., Osman A. M. M., & Abdel-Sattar E. A. (2011). In vitro cytotoxic screening of selected Saudi medicinal plants. *Journal of Natural Medicine, 66*, 406–412.

Al-Snafi, A. E. (2016). A review on *Cyperus rotundus* a potential medicinal plant. *IOSR Journal of Pharmacy*, *6*(7), 32-48

Amala, V. E., & Jeyaraj, M. (2014). Comparative evaluation of phytocomponents present in the methanolic extract of *Terminalia chebularetz, Terminalia bellirica roxb* and *Phyllanthus emblica* L., fruit extracts using GC-MS analysis. *International Journal of Pharmacy and Biological Sciences*, *5*(4), 927-934

Andarge, E., Shonga, A., Agize, M., & Tora, A. (2015). Utilization of medicinal plants and their associated indigenous knowledge (Ik) in Dawuro zone: An ethnobotanical approach. *International Journal of Plant Research*, *4*(3), 330-337.

Araya, S., Abera, B., & Giday, M. (2015). Study of plants traditionally used in public and animal health management in Seharti Samre District, Southern Tigray, Ethiopia. *Journal of Ethnobiology and Ethnomedicine, 10,* 11-22.

Archana, R., Kanchana, G., & Rubalakshmi, G. (2014). Identification of bioactive compounds from marine sponge –sporangia tost by GC-MS analysis. *World Journal of Pharmacy and Pharmaceutical Sciences*, *3*(11), 439-445.

Arora S., & Kumar, G. (2017). Gas Chromatography-Mass Spectrometry (GC-MS) determination of bioactive constituents from the methanolic and ethyl acetate extract of *Cenchrus setigerus* Vahl (Poaceae). *Pharma Innovation Journal*, *6*(11), 635-640

Arunkumark, V., & Paridhavi, M. (2013). Evaluation of the components and antimicrobial activity of volatile oil from *Zanthoxylumlimonella* fruit. *International Journal of Pharmacy and Biological Sciences*, *4*(2), 777-787

Arunmathi, C. and Malarvili, T. (2017). Analysis of bioactive compounds in methanol extract of *Aplotaxis auriculata* rhizome using GC-MS. *Journal of Pharmacognosy and Phytochemistry*, *6*(3), 243-247.

Asfaw, Z. (2001). The role of home garden in production and conservation of medicinal plants. Conservation and sustainable use of medicinal plants in Ethiopia. *Institute of Biodiversity Conservation and Research, Addis Ababa, 9*, 76-91

Ashwini, U., & Asha, S. (2017). Larvicidal activity of natural products against mosquito species. A review. *International Journal of Chemistry and Technological Research. 10*(5), 875-878.

Asnake, S., Teklehaymanot, T., Hymete, A., Erko, B., & Giday, M. (2016). Survey of medicinal plants used to treat malaria by Sidama people of Boricha district, Sidama zone, South region of Ethiopia. *Hidawi Publishing Corporation, 9*, 33-49

Azevedo, M. B., Chaves F. M., Almeida C. A., Bizzo H. R., Duarte R. S., Campos-Takaki, G. M., … Alviano, D. S. (2013). Antioxidant and Antimicrobial Activities of 7-Hydroxycalamenene-Rich Essential Oils from Croton cajucara Benth. *Molecules, 18*, 1128-1137.

Bagavan, A., & Rahuman, A. A. (2011). Evaluation of larvicidal activity of medicinal plant extracts against three mosquito vectors. *Asian Pacific Journal of Tropical Medicine, 7*, 29-34

Curvelo, J. R., Marques, A. M., Barreto, A. S., Romanos, M. V., Portela, M. B., Kaplan, M. C., & Soares, R. A. (2014). A novel Nerolidol-rich essential oil from Piper claussenianum modulates *Candida albicans* biofilm. *Journal of Medical Microbiology*, *63*, 697-702.

Damasceno, C. S. B., Letícia, O. F., Szabo, E. M., Souza, A. M., Dias, J. F. G., Miguel, M. D., & Miguel, O. G. (2017). Chemical composition, antioxidant and biological activity of *Ocotea bicolor* Vattimo-Gil (LAURACEAE) essential oil. *Brazilian Journal of Pharmaceutical Sciences, 53*(4), 1-8.

Dambolena, J. S., Zunino, M. P., Herrera, J. M., Pizzolitto, R. P., Areco, V. A., & Zygadlo, J. A. (2016). Terpenes: Natural products for controlling insects of importance to human health –A stracture –activity relationship study. *Hidawi Publishing Corporation Psyche, 10*, 1- 17

Dame, D., Wichterman, G., & Hornby, J. (1998). Mosquito (*Aedes taeniorhynchus*) resistance to methoprene in an isolated habitat. *Journal of Mosquito Control Association, 14*, 200-203.

Danis, K., Lenglet, A., Tseroni, M., Baka, A., Tsiodras, S., & Bonovas, S. (2013). Malaria in Greece: Historical and current reflections on a re-emerging vector borne disease. *Travel Medicinal Infectious Diseases, 11*, 8 - 14.

Dias, C. N., Alves, L. P. L., Rodrigues, K. A. F., Brito, M. C. A., Rosa, C. S., Amaral, F. M., & Moraes, D. F. C. (2015). Chemical Composition and Larvicidal Activity of Essential Oils Extracted from Brazilian Legal Amazon Plants against *Aedes aegypti* L. (Diptera: Culicidae). *Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine, 10*, 1-8.

Dinesh, K. G., & Rajakumar, R. (2016). Gas chromatography mass spectrometry analysis of bioactive components from the ethanol extract of *Avicennia marina* leaves. *Journal of Science, 4*, 9-12.

Dua, V. K., Pandey, A. C., & Dash, A. P. (2010). Adulticidal activity of essential oil of *Lantana camara* leaves against mosquitoes. *Indiana Journal of Medical Research, 131*, 434-439

Elangovan, A., Veeraiyan, G., Elumalai, K., & Prakash, M. (2008). Larvicidal Activity of plant oil formulation against three important vector mosquito species. *Journal of Veterinary Medicine, 6*(1), 39-53.

Equar, G., Abraha, B., Lemma, H., Amare, S., & Asmelash, T. (2016). Honey bee flora diversity and their impact on honey production in Tigray region of Ethiopia. *Livestock Research for Rural Development Journal, 28*, 122-137.

Erturk, O., Sekeroglu. V., & Kalkan, A. K. Y. (2004**).** Antifeedant and toxicity effects of some plant extracts on Yponomeuta malinellus zell. (Lep: Yponomeutidae). *Journal of Plant Protection Research, 44*(3), 165-174.

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

Goselle, O. N., Gyang, D. A., Adara, O. F., Eftiong, K. T., Nanvyat, N., Adulungba, I. A., … Mafuyai, H. B. (2017). A comparative study of the larvicidal activity of lemongrass (Cymbopogan citrates) from different methods of extraction. *Journal of Academia and Industrial Research, 6*(3), 47-53.

Govindarajulu, B., Srimathi, A., Bhuvana R., & Karthikeyan, J. (2015). Mosquito Larvicidal Efficacy of the Leaf Extracts of *Annona reticulate* Against *Aedes aegypti. International Journal of Microbial Applied Sciences, 4*(8), 132-140

Grigoraki, L., Balabanidou, V., Pipini, D., Strati, F., & Vontas, J. (2016**).** Analysis of insecticide resistance in mosquito disease vectors: From molecular mechanisms to management. *Nova Acta Leopoldina Journal, 411*, 165 –171.

Gutierrez, P. M, Antepuesto, J. N., Eugenio, A. L., & Santos, M. F. L. (2014). Larvicidal Activity of Selected Plant Extracts against the Dengue vector *Aedes aegypti* Mosquito. *International Research Journal of Biological Science, 3*(4), 23-32.

Haftom, G., & Yaynishet, T. (2012). Identification and evaluation propagation techniques of *Hypoestes forskaolii* (Grbia) as bee fodder for small holder farmers. *Livestock Research for Rural Development, 24*, 134-169.

Hemalatha, P., Elumalai, D., Janaki, A., Babu, M., Velu, K., Velayutham, K., & Kaleena, P. K. (2015). Larvicidal activity of Lantana camara aculeate against three important mosquito species. *Journal of Entomology and Zoology Studies, 3*(1), 174-181.

Isijola, A. O. M., Olajuyigbe, O. O., Jonathan, G. S., & Coopoosamy, M. R. (2018). Bioactive compounds in ethanol extract of *Lentinus squarrosulus* mont - a Nigerian medicinal macrofungus. *African Journal of Traditional Complement Alternative Medicine*, *15*(2), 42-50

Jain, S. C., Pancholi, B., & Jain, R. (2012). Antimicrobial, free radical scavernging activities and chemical composition of *Peltophorum pterocarpum* Backer ex K. Heyne stem extracts. *Dermatology Pharmaceutical Chemica*, *4*(5), 2073-2079.

Jananie, R. K., Priya, V., & Vijayalakshmi, K. (2011). Determination of bioactive components of *Cynodon dactylon* by GC-MS Analysis. *New York Science Journal*, *4*(4), 16-20.

Jarsso, B. (2016). Ethnobotanical study of traditional medicinal plants used by indigenous people of Jigjiga Werenda Somali regional state, Ethiopia. A thesis submitted to the department of Biology, College of Natural and Computational Sciences, Haramay University. pp. 1-64

Jayapriya, G., & Griselda, S. F. (2015). Adulticidal and Repellent activities of *Rhinacanthus nasulus* leaf extracts against *Aedes aegypti* Linn and *Culex quinquefasciatus* Say. *Journal of Entomology and Zoology Studies*, *3*(1), 154-159.

Kabaru, J. M., & Gichia, L. (2001). Insecticidal activity of extracts derived from different parts of the Mangrove tree *Rhizophora mucronata* (Rhizophoraceae) Lam. against three arthropods. *African Journal of Science and Technology, Science and Engineering Series*, *2*(2), 44-49.

Kamundi, D. A. (2006). Hypoestes *forskaolii* (Vahl) R. Br. National Assessment: *Red List of South African Plants Version* 2017.1. Accessed on 2018/07/26.

Karanja, E., Boga, H., Muigai, A., Wamunyokoli, F., Kinyua, J., & Nonoh, J. (2012). Growth characteristics and production of secondary metabolites from selected novel *Streptomyces* species isolated from selected Kenyan national parks. *Frontiers in Microbiology*, *7*, 1-10.

Kareu, P., Rotich, Z. K., & Maina, E. W. (2013). Use of botanicals safer insecticides designed in controlling insects the African case. [Http://dx.doi.org/10.5772/5324]. Accessed on 2018/7/26.

Khater, H. F. (2012). Prospects of botanical biopesticides in insect pest management. *Pharmacologia*, *3*(12), 641-656.

Kidane. B., Andel, T. V., Josephus, L., Maesen, G. V., & Asfaw, Z. (2014). Use and management of traditional medicinal plants by Maale and Ari ethnic communities in southern Ethiopia. *Journal of Ethnobiology and Ethnomedicine, 10*, 1- 46

Kilonzo, M., Ndakidemi, P. A., & Chacha, M. (2017). *Mystroxylon aethiopicum* chloroform root bark extracts phytochemical analysis using gas chromatography mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*, *9*(4), 44-50.

Kipkore, W., Wanjohi, B., Rono, H., & Kigen, G. (2014). A study of the medicinal plants used by the Marakwet Community in Kenya. *Journal of Ethnobiology and Ethnomedicine, 10*, 1-24.

Komalamisra, N., Trongtokit, Y., Rongsriyam, Y., & Apiwathnasorn, C. (2005). Screening for larvicidal activity in some Thai plants against four mosquito vector species. *Southeast Asian Journal Tropical Medical Public Health*, *36*(6), 12-22

Kumar, S., Wahab, N., Mishra, M., & Warikoo, R. (2012). Evaluation of 15 local plant species as larvicidal agents against an Indian strain of dengue fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae). *Frontiers in Physiology*, *10*, 33-89.

Leandro, L. M., Fabiano, S. V., Paula, C. S. B., Jamilly, K. O. N., José A. S., & Valdir, F. V. J. (2012). Chemistry and Biological activities of Terpenoids from Copaiba (*Copaifera* spp.) Oleoresins. *Molecules, 17*, 3867-3889

Lee, S. E. (2000). Mosquito larvicidal activity of pipernonaline piperidine alkaloid derived from long pepper piper longum. *Journal of the American Mosquito Control Association, 16*(3), 245-247.

Lin, J., Dou, J., Xu, J., & Aisa, H. A. (2012). Chemical composition, antimicrobial and antitumor activities of essential oils and crude extracts of *Euphorbia macrorrhiza*. *Molecules, 17*, 5030-5039

Magalhães, L. A. M., Lima, M. P., Marques, M. O. M., Facanali, R., Pinto, A. C. S., & Tadei, W. P. (2010). Chemical composition and larvicidal activity against *Aedes aegypti* larvae of essential oils from four *Guarea* species. *Molecules, 15*, 5734-5741.

Makirita, W., Chauka, L., & Chacha, M. (2015). Larvicidal activity of *Clausena anisata* fruits and leaves extracts against *Anopheles gambiae Giless, S., Culex quinquefasciatus Say* and *Aedes aegyptiae*. *Spatula DD, 5*(3), 147-153.

Malarvilli, A. T. (2017). Analysis of bioactive compounds in methanol extracts of *Apcotaxis curiculata* rhizome using GC-MS. *Journal of Pharmacognosy and Phytochemistry, 6* (3), 243-247.

Mathew, J., & Thoppil, J. E. (2010). Chemical composition and mosquito larvicidal activities of *Salvia* essential oils. *Pharmaceutical Biology, 49*(5), 456-463.

Mavundza, E. J., Maharaj, R., Chukwujekwu, J. C., Finnie, J. F., & Staden, J. V. (2014). Screening for adulticidal activity against *Anopheles arabiensis* in ten plants used as mosquito repellent in South Africa. *Malaria Journal, 10*, 13-173.

Meenakshi, V. K., Veerabahu, C., & Roselin, K. F. (2013). GC-MS and IR studies of ethanolic extract of colonial ascidians- *Polyclinum madrasensis* Sebastian, 1952. *International Journal Pharmacology Biological Science, 4*(4), 1187-1198.

Merritt, R. W., Dadd, R. H., & Walker, E. D. (1992). Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annual Review of Entomology, 37*, 349-76.

Mohammed, G. J., Omran, A. M., & Hussein, H. M. (2016). Antibacterial and phytochemical analysis of *Piper nigrum* using gas chromatography – mass spectrum and fourier – transform infrared spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*, *8*(6), 977-996.

Mukhatar, M. U., Mushtag, S., Zaki, A. A. B., Hammad, M., & Bhatti, A. (2015). Laboratory study on larvicidal activity of different plant extracts against *Aedes aegypti*. *International Journal of Mosquito Research*, *2*(3), 127-130.

Murugesan, S., Rajeshkannan, C., Babu, D. S., Sumathi, R., & Manivachakam, P. (2012). Identification of insecticidal properties in common weed-Lantana camara Linn by Gas chromatography and mass spectrum (GC-MS). *Advances in Applied Science Research*, *3*(5), 2754-2759

Murugesan, S., Rajeshkannan, C., Sumathi, R., Manivachakam, P., & Babu, D. S. (2012). Bioactivity of root hexane extract of *Coelus forskaolii* Brig. Labiatae: GC/MS/MS characterization and identification. *Europian Journal of Experimental Biology*, *2*(5), 1469-1473

Musayeib, N. M. A., Mothana, R. A., Mohamed, G. A., Ibrahim, S. R. M., & Maes, L. (2014). Hypoestenonols A and B, new fusicocane diterpenes from *Hypoestes forskalei*. *Phytochemistry Letters*, *10*(1), 23-27.

Nandhini, S. U. (2015). Gas chromatography–mass spectrometry analysis of bioactive constituents from the marine *Streptomyces*. *Asian Journal Pharmacology Clinical Research, 8*, 244–246.

Ntalli, N. G., & Spiroudi, U. M. (2010). Pesticides of Botanical Origin: a promising tool in plant protection, pesticides - formulations, effects, fate. *Prof. Margarita Stoytcheva Education, 7*, 307-532.

Otitolaiye, C. A., & Asoken, C. (2016). GC-MS analysis of *Cnidoscolus aconitifolius* leaf aqueous extracts. *International Journal of Science and Research*, *6*, 2319-7064.

Parera, A., Karunaratne, M., & Chinthaka, S. (2017). Biological activity and secondary metabolite profile of Ruta graveolens leaves against maize weevil infestation. *Journal of Entomology and Zoology Studies, 5*(2), 233-241.

Pavela, R. (2016). History, Presence and perspectives of using plant extracts as commercial botanical insecticides and farm products for protection against insect – a review. *Plants protection Science*, *52*(4), 229-241.

Polanco, H. G., Escalante, E. F., García, S. K., María, E. R., Eugenia, G. M., Karla, Y., & Acosta, V. (2015). Synergistic Effect of Lupenone and Caryophyllene Oxide against *Trypanosoma cruzi*. *Evidance Based Complement and Alternative Medicine*, *10*, 1-6.

Prabodh, S., & Setzer, W. N. (2014). Chemotyping and determination of antimicrobial, insecticidal, and cytotoxic properties of wild-grown cannabis sativa from Nepal. *Journal of Medicinally Active Plants, 3*(4), 9-16

Prakasia, P. P., & Nair, A. S. (2015). Chemical fingerprint of essential oil components from fresh leaves of *Glycosmispentaphylla* (Retz.) Correa. *The Pharma Innovation Journal, 3*(12), 50-56.

Raj, G. A., Chandrasekaran, M., Krishnamoorthy, S., Jayaraman, M., & Venkatesalu V. (2015). Phytochemical profile and larvicidal properties of seed essential oil from *Nigella sativa* L. (Ranunculaceae), against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research*, *10*, 1- 100.

Rajeswari, J., & Rani, S. (2015). GC-MS analysis of phytochemical compounds in the ethanolic extract of root of *Lawsonia inermis* Linn. *International Journal of Chemistry and Technological Research, 7*(1), 389-399.

Rajeswari, N., RamaLakshmi, S., & Muthuchelian, K. (2011). GC-MS analysis of bioactive components from the ethanolic leaf extract of *Canthium dicoccum*. *Journal of Chemistry and Pharmaceutical Research, 3*(3), 792-798.

Ramar, M., Bcimuthu, S. I., & Paulraj, M. G. (2014). Mosquito knock-down and adulticidal activities of essential oils by vaporizer, impregnated filter paper and aerosol methods. *International Journal of Mosquito Research*, *1*(3), 26-32.

Ranasinghe, M. S. N., Arambewela, L., & Samarasinghe, S. (2016). Development of herbal Mosquito repellent formulations. *International Journal of Collaborative Research on Internal Medicine and Public Health, 8*(6), 341-380.

Ranson, H., Burhani, J., Lumjuan, N., & Black, W. (2010). Insecticide resistance in dengue vectors. *Tropika.network, 1*(1), 1-12

Ranson, H., N'Guessan, R., Lines, J., Moiroux, N., Nkuni, Z., & Corbel, V. (2011). Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control. *Trends Parasitology*, *27*, 91–98.

Rathy, M. C., Sajith, U., & Harilal, C. C. (2014). Plant diversity for mosquito control: A preliminary study. *International Journal of Mosquito Research, 2*(1), 29-33

Raymond, M., Berticat, C., Weill, M., Pasteur, N., & Chevillon, C. (2001). Insecticide resistance in the mosquito *Culex pipiens*: what have we learned about adaptation. *Genetica*, *112*, 287-296.

Regnault-Roger, C. (1997). The potential of botanical essential oils for insect pest control. *Integrated Pest Management Review*, *2*, 25-34.

Rehman, J. U., Jilani, G., Khan, M. A., Masin, R., & Kanvil, S. (2009). Repellant and oviposition deterrent effects of indigenous plant extracts to peach fruit fly, Bactrocera zonata Saunders (Diptera: Tephritidae). *Pakistan Journal of Zoology*, *41*(2), 101-108.

Rency, R. C., Vasantha, K., & Maruthasalam, A. (2015). Identification of bioactive compounds from ethanolic leaf extracts of *Premna serratifolia* L. Using GC-MS. *Bioscience Discovery Journal*, *6*(2), 96-101.

WHO. (1996). Informal consultation on the Evaluation and Testing of Insecticides, WHO pesticides evaluation scheme (WHOPES) world health organization division of control of tropical diseases (CTD), WHO, Geneva 7-11 October 1996,CTD/ WHOPES/IC/96.1

Romeh, A. (2013). Phytochemicals from *Ficus sycomorus* L. Leaves act as insecticides and acaricides. *African Journal of Agricultural Research, 8*(27), 3571-3579.

Rose, R. I. (2001). Pesticides and Public Health: Integrated Methods of Mosquito Management. *Emerging Infectious Diseases*, *7*(1), 17-23.

Rukangira, E. (2001). Medicinal plants and Traditional medicine in Africa: Constraints and Challenges. *Sustainable Development International Journal*, *4*, 179-184.

Sahi, N. (2016). Evaluation of insecticidal activity of bioactive compounds from Eucalyptus citrodora against Tribolium. *International Journal of Pharmacognosy and Phytochemical Research, 8*(8), 1256-1270.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Yoga, L. L. (2011). Extraction, isolation and characterization of bioactive compound from plant extracts. *African Journal of Traditional Compliment Alternative Medicine, 8*(1), 1-10.

Saxena, R. C. (1987). Antifeedant in Tropical pest management, *Insect Science Application, 8*, 731-736

Okwute, S. K. (2012). Plants as Potential Sources of Pesticidal Agents: A Review. *Pesticides – Advances in Chemical and Botanical Pesticides*, *10*, 208-232.

Siqueira, J. M., Ziminiani, M. G., Resende, U. M., & Boaventura, M. A. D. (2001). Activity-guided isolation of the constituents from bark of stem of *Duguetia glabriuscula* - Annonaceae, using brine shrimp lethality test (BSL*). Quim Nova Journal*, *24*, 185–187.

Sivasubramanian, R., & Brindha, P. (2013). Invitro cytotoxic, antioxidant and GC-MS studies on *Centrutum punctatum* Cass. *International Journal of Pharmacy and Pharmaceutical Science, 5***(**3), 975-1491

Sneddon, J., Masuram, S., & Richert, J. C. (2007). GC-MS-Basic principles, instrumentation and selected applications for detection of organic compounds. *Analytical Letters, 40*(6), 1003-1012.

Soetan, K. O., & Aiyelaagbe, O. O. (2009). The need for bioactivity safety evaluation and conservation of medicinal plant. A Review. *Journal of Medicinal Plant Research, 3*(5), 324-328.

Solis, C., Becerra, J., Flores, C., Robledo, J., & Silva, M. (2004). Antibacterial and antifungal Terpenes from *Pilgerodendron uviferum* (D. Don) Florin. *Journal Chilean Chemistry Societies, 49*(2), 157-16

Subramaniam, J., Murugan, K., & Kovendan, K. (2012). Larvicidal and pupcidal efficacy of *Momordica charantia* leaf extract and bacterial insecticide, *Bacillus thuringiensis* against malarial vector, *Anopheles stephensi* Liston. (Diptera: Culicidae). Botanical and bacteria for vector control. *Journal of Biopesticide, 5*, 163-169.

Subramanian, J., Kovendan, K., Kumar, P. M., Murugan, K., & Walton, W. (2012). Mosquito larvicidal activity of Aloe vera (Family: Liliaceae) leaf extract and *Bacillus sphaericus*, against Chikungunya vector, Aedes aegypti. *Saudi Journal of Biological Sciences, 10*, 10-16

Sujayil, T. K., & Dhanaraj, T. S. (2016). Determination of bioactive compounds in *Evolvulus alsinoides* leaf extracts using GC-MS technique**.** *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences, 2*(3), 31-34.

Teklay, A., Abera, B., & Giday, M. (2013). An ethnobotanical study of medicinal plants used in Kilte Awulaelo district, Tigray region of Ethiopia. *Journal of Ethnobiology and Ethnomedicine, 9*, 1-65.

Teklay, A. (2015). Traditional medicinal plants for ethnoverternary medicine used in Kilte Awulaelo district, Tigray region, Northern Ethiopia: *Advancement in Medicinal Plant Research*, *3*(4), 137-150.

The report of WHO. (2003). The African Malaria Report. WHO/CDS/MAL.1903, 31-37

Tyagi, T., & Agarwal, M. (2017). Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistiastratiotes* L. and *Eichhornia crassipes* (Mart.) solms. *Journal of Pharmacognosy and Phytochemistry*, *6*(1), 195-206

Vijisaral, E. D., & Arumugam, S. (2014). GC-MS analysis of bioactive constituents of *Indigofera suffruticosa* leaves. *Journal of Chemical and Pharmaceutical Research*, *6*(8), 294-300

Vontas, J., Kioulos, E., Pavlidi, N., Morou, E., Torre, D. A., & Ranson, H. (2012). Insecticide resistance in the major dengue vectors *Aedes albopictus* and *Aedes aegypti*. *Pesticides Biochemical Physics, 104*, 126 –131.

Warikoo, R., Ray, A., Sandhu, J. K., Samal, R., Wahab, N., & Kumar, S. (2012). Larvicidal and irritant activities of hexane leaf extracts of tropical biomedicine. *Asian Pacific Journal of Tropical Biomedics*, *2*(2), 152-155.

Chen, W., Lan, Z., Weijun, C., Hu, Y., & Chen, H. (2018). Effects of black Pepper *(Piper nigrum L.)* chloroform extract on the enzymatic activity and metabolism of Escherichia *coli* and *Staphylococcus aureus. Hindawi Journal of Food Quality, 10*, 1-9.

WHO. (1996). Informal Consultation on the Evaluation and Testing of Insecticides. (1996: Geneva, Switzerland), World Health Organization. Division of Control of Tropical Diseases and WHO Pesticide Evaluation Scheme. (1996). Report of the WHO Informal Consultation on the "Evaluation and Testing of Insecticides", WHO/HQ, Geneva, 7 to 11 October 1996. Geneva: World Health Organization. http://www.who. int/iris /handle/10665 /65962

WHO. (1997). Vector control methods for use by individuals and communities. WHO Pesticide Evaluation Scheme (WHOPES) ISBN 9241544945.

Zeeshan, M., Rizvi, S. D., Khan, M. S., & Kumar, A. (2012). Isolation, partial purification and evaluation of bioactive compounds from leaves of *Ageratum houstoniamnum*. *EXCL. Journal*, *11*, 78-88.

Zekeya, N., Chacha, M., Shahada, F., & Kidukuli, A. (2014). Analysis of phytochemical composition of *Bersama abyssinica by* gas chromatography – mass spectrometry. *Journal of Pharmacognosy and Phytochemistry*, *3*(4), 246-252.

Zhang, J., Dou, J., Zhang, S., Liang, Q., & Meng, Q. (2010). Chemical composition and antioxidant properties of the essential oil and methanol extracts of rhizoma *Alpinia officinarum* from China in vitro. *African Journal of Biotechnology*, *9*(28), 4414-442.