LARVICIDAL EFFICACY OF ETHANOIC EXTRACT OF LEAF AND BARK OF CHRYSOPHYLLUM ALBIDUM AGAINST CULEX SPECIES

BY

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DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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A DISSERTION SUBMITTED TO THE DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY, IN PARTIAL FUFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (BSC.HONS) IN ANIMAL AND ENVIRONMETAL BIOLOGY, OF THE UNIVERSITY OF BENIN, BENIN CITY.

DECEMBER, 2022

CERTIFICATION

We certify that this project was done by **EBIMIYENERE SUCCESS ITIKPAN**

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DEDICATION

This project work is dedicated to the God Almighty for giving me the wisdom, strength and guidance to successfully carry out this research work. This work is also dedicated to my parents for their financial and moral support throughout my stay at the university and especially during my research period.

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ABSTRACT

Botanical insecticides may serve as suitable alternatives to synthetics in future, as they are relatively safe and readily available in many parts of the world. This study investigated the efficacy of *Chrysophyllum albidum* extracts (Leaf and Bark stem) on *Culex* species larvae. The extracts were tested on laboratory bred Culex sp. larvae at different concentrations (500, 750 and 1000ppm) at 24, 48 and 72 hrs. Data was analyzed statistically using Analysis of Variance (ANOVA). Increasing mortalities was observed with increase in concentration and time of exposure. Highest mortalities of *Chrysophyllum albidum* bark per exposure time were observed in larvae exposed to highest test concentration of 1000 ppm (24 h = 3.3%; 48 h = 10.0%; 72 h = 13%). Highest mortalities of *C. albidum* leaf per exposure time were observed in larvae exposed to highest test concentration of 1000 ppm (24 h = 10%; 48 h = 16.7 %; 72 h = 23.3%). LC50 and LC90 values at 72 hrs were 6332.262 ppm and 58278.321 ppm respectively for *Chrysophyllum albidum* bark, 4672.394 ppm and 46866.555 for the leaf and 4265.617ppm and 42292.050 for both the leaf and bark. The phytochemicals screening of ethanoic extract of *Chrysophyllum albidum* from both the leaves and bark revealed the presence of carbohydrate, saponins, tannins, phenol, steroid and alkaloids in *Chrysophyllum albidum* and the absence of phenol in leaves. The findings of the present investigation revealed that the leaf extract of *Chrysophyllum albidum* showed larvicidal activity against *Culex* sp. Chemicals derived from plants offer promise in future mosquito control programs.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Mosquito (Diptera: Culicidae) presents an array of insects which more than any other group poses the greatest challenge to human and veterinary health as vectors of diseases (Guzman *et al.,* 2010). Worldwide, mosquitoes are a major public health problem. They are estimated to transmit diseases to more than 700 million people annually and are predicted to be currently responsible for the deaths of about one in 17 people (WHO, 2005).

Mosquitoes are found in different areas including moist areas such as stagnant water, slow flowing water, flowing water with several blockages such as wastes and macrophytes and dirty environments. In Nigeria mosquitoes are common in the area with large surface water (creek, creeklets, stream, pond, rivers). Mosquitoes constitute a nuisance during sleeping due to the noise they make in addition to blood sucking. The biting is usually intense around 6 to 7 am, with maximum intensity between 10 pm and 4 am under the Nigerian climatic conditions (Aziz *et al.,* 2014).

Diseases transmitted by mosquitoes are prevalent in more than 100 countries around the world, infecting over 700 million people globally each year (WHO, 2005). On the basis of public health importance, they form the most important group of insects that transmit a number of diseases such as malaria, filariasis, Japanese encephalitis, dengue, and chikungunya. Nigeria suffers the world's greatest malaria burden, with approximately 51 million cases and 207,000 deaths

reported annually while 97% of the total population is at risk of infection (WHO, 2014). Yellow fever epidemics have also had devasting effects on human population (Nnasidi et al.,1989).

Culex mosquitoes (Diptera: Culicidae) are pantropical pests that are likely the most abundant house mosquitoes prevailing in urban and rural areas (Wajiha *et al*., 2017). *Culex* sp. is one of the most dominant mosquito species in Nigeria (Otabor *et al.,* 2019). It is a vector of filarial fever and encephalitis, and it is a major public health concern in many developing countries (Arivoli *et al.,* 2011; Ashfaq and Ashfaq, 2012). One of the major strategies is to control the vector or immediate host, which can minimize the spread of disease, and this strategy may be applied against immature or adult insect stages

Synthetic insecticides can be harmful to the environment and natural enemies (Rocha et al., 2006), making it necessary to select those which are effective, safe, and selective for pest control. Botanical extracts are a potentially valuable alternative method of controlling insect pests as they have lower persistence and toxicity than synthetic insecticides (Hossain and Poehling, 2006). These natural insecticides possess secondary compounds such as terpenoids that protect plants by causing various effects in insects, including behavioral and physiological responses (Tedeschi *et al.,* 2001), but may have adverse effects on natural enemies (Coley *et al.,* 2006). These substances may be more harmful to generalist predators than to pests (Coley *et al*., 2006). The plant families containing the most promising botanical insecticides are Annonaceae, Asteraceae, Canellaceae, Lamiaceae, Meliaceae, and Rutaceae (Isman 2006). The use of plant extracts in pest control is increasing due to consumer demand for pesticide-free products (Isman, 2006). However, few studies have demonstrated the impact of botanical extracts on natural enemies, thus increasing the need to study their effects on beneficial organisms.

Botanical insecticides may serve as suitable alternatives to synthetics in future, as they are relatively safe, easily degradable and readily available in many parts of the world (Sivagnaname and Klyanasundaram, 2004). Although bio-pesticides of plant origin have been extensively used on agricultural pest control, a very limited extent has been used against insect vectors of public health importance (Das *et al*., 2007). Because of these, many of the reported tropical plants came under scrutiny, leading to extraction and characterization of their active ingredients. Among the most important plant constituents are alkaloids, terpenoids, steroids, phenols, saponins and tannins (Shaalan *et al.,* 2005). Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents, oviposition deterrents and affect different activities of the organisms (Eich, 2008).

1.2 JUSTIFICATION OF STUDY

Although several compounds of plant origin have been reported as bio-pesticides and used for control of mosquito larvae, there is still a wide scope for the discovery of more effective plant products (Saxena and Yadav, 1986) particularly in the indigenous flora of lesser studied countries like Nigeria.

This study investigated the larvicidal efficacy of the ethanoic extract of *Chrysophyllum albidum* bark and leaf.

1.3 AIM AND OBJECTIVES

The Aim of this Study was to investigate the Efficacy of *Chrysophyllum aldidum* Extracts (Leaf and Bark stem) on *Culex* species larvae.

The specific objectives are to:

1. Determine the larvicidal activity of leaf and stem bark extract of *Chrysophyllum aldidum* on *Culex* species
2. Determine the phytochemical content of *Chrysophyllum aldidum* extracts (leaf and stem bark)
3. Assess the larvicidal potency of the phytochemicals of *Chrysophyllum aldidum* extracts on *Culex* sp.

CHAPTER TWO

LITERATURE REVIEW

According to Richa *et al.,* (2017) for effective control of mosquitoes, research should focus on smart and innovative techniques to control mosquitoes which involve the understanding of the fundamental biology and physics of the vector. A review on the medicinal plants with antimalarial properties in Nigeria has been comprehensively documented by Adebayo and Krettli (2018). Therefore, this review focuses on the various Nigerian plants that have insecticidal potentials against mosquitoes.

Arthropods are dangerous vectors of deadly pathogens and parasites, which may spread as epidemics or pandemics in the increasing world population of humans and animals (Mehlhorn, 2008, Mehlhorn *et al.,* 2012). Mosquitoes transmit a variety of parasitic (e.g., malaria and lympatic filariasis) and viral (e.g., dengue, zika, and yellow fever) diseases that pose serious public health challenge worldwide. The three genera of mosquitoes are *Culex*, *Anopheles*, and *Aedes* which transmit major mosquito based vector-borne diseases.

2.1 DESCRIPTION OF CULEX SPECIES

The adult *C. species* is a medium-sized mosquito and is brown in colour. The body is about 3.96 to 4.25 mm long (Arivoli *et al.,* 2011). While the main body is brown, the proboscis, thorax, wings, and tarsi are darker than the rest of the body. The head is light brown, with the lightest portion in the center (Mehlhorn *et al.,* 2012). 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 13 segments that may have few or no scales. The scales of the thorax are narrow and curved. The abdomen has pale, narrow, rounded bands on the basal side of

each tergite. Males can be differentiated from females in having large palps and feathery antennae (Guzman *et al.,* 2010).

The larva has a short and stout head. The mouth brushes have long yellow filaments used for filtering organic materials. The abdomen consists of eight segments, the siphon, and the saddle. Each segment has a unique setae pattern (Weston, 2005). The siphon is on the dorsal side of the abdomen, and is four times longer than its breadth. The siphon has multiple setae tufts. The saddle is barrel-shaped and located on the ventral side of the abdomen, with four long anal papillae protruding from the posterior end (Aziz *et al.,* 2014).

2.2 CHRYSOPHYLLUM ALBIDUM

Chrysophyllum albidum fruit, known locally as Agbalumo or Udara in Nigeria, has fleshy pulp which is widely consumed when in season by both adult and children, with either a very sweet taste (when fully ripe), or sour (when unripe). The plant, a forest tree, common throughout the tropical African, belongs to the family Sapotaceae. *Chrysophyllum albidum* fruits (known as African star apple) are widely eaten in southern Nigeria. The fruit is seasonal (December-March), when ripe, ovoid to sub-globose, pointed at the apex, and up to 6 cm long and 5 cm in diameter. The skin or peel, is orange to golden yellow when ripe and the pulp within the peel may be orange, pinkish, or light yellow, within the pulp are three to five seeds which are not usually eaten. The seed-coats are hard, bony, shiny, and dark brown, and when broken reveal white-coloured cotyledons. The fruit has immense economic potential, especially following the report that jams that could compete with raspberry jams and jellies could be made from it (Okafor, 1975). The fleshy fruit pulp is suitable for jams and is eaten especially as snack by both young and old (Amusa *et.al.,* 2003).

Chrysophyllum albidum Is a rich source of natural antioxidant such as flavonoids and vitamin E, C and A. They prevent oxidation of heart health. It helps to manage and control diabetes, evidence suggests that African star apple pulp can lower blood sugar levels and act as an effective dietary supplement to manage diabetes.

Oputah *et al*., (2016) studied the phytochemicals of the seeds of African Star Apple (*Chrysophyllum albidum*) from their study, the phytochemical result revealed the presence of saponins, carbohydrates, flavonoids, quinones, cardiac glycosides, fatty acids and terpenoids.

2.3 PHYTOCHEMICAL AND PLANTS

Phytochemicals are defined as bioactive nutrient plant chemicals in fruits, vegetables, grains, and other plants foods that may provide desirable health benefits beyond basic nutrition to reduce the risk of major chronic diseases (Liu, 2004).

Weeds are enemies to crop plants and have harmful effects on agricultural crops due to several factors such as competition for space, light and nutrients. Organic chemicals released as leaf leachates, affect crop plants. Weeds species are considered as rich source of secondary metabolites (allelochemicals). These chemicals improve a certain kind of environmental system on other plants growing in their vicinity and this phenomenon is known as allelopathy (Nandal *et al.,* 1994). Weston (2005) defined allelopathy as an important mechanism of plant interference mediated by the addition of plant secondary products to the soil rhizosphere. These secondary metabolites are located throughout the plant and are present in various plant tissues such as stems, leaves, roots and others. In agriculture, the inhibitory effect of weed species on germination and growth of crops has been attributed to phytotoxic chemicals released from the leaf litter and roots.

Romero-Romero *et al.* (2005) reported that the effect of such compounds is harmful to plant growth and development and it may become a biotic or allelochemical stress.

2.4 TOXICITY OF PHYTOCHEMICALS ON MOSQUITOES

Toxicity of phytochemicals in mosquitoes was first reported by Campbell *et al.* (1933). In a review paper, Sukumar *et al.* (1991) summarized a list of 104 (out of 344 tested) plant species from 49 families that possessed either larvicidal, pupicidal, and/or adulticidal activity on *Aedes aegypti* L. Recent papers from all over the world have documented the toxic effect of plant extracts on the mosquito larvae (Tare *et al.,* 2004, De Lima *et al.,* 2006, Promsiri *et al.,* 2006), but most species of plants from Nigeria have not yet been examined for its activity on *C. species*. More than 1,000 species of plants are used for the treatment of human diseases, but less than 20% of all plant species have been investigated (García-Alvarado *et al.,* 2001).

Okbatinsae and Haile (2016) studied the larvicidal effects of plant extracts against larvae of *Anopheles gambiae* in the malaria entomology laboratory at Mendefera. The aim of the study was to evaluate ethanol and hot water extracts from leaves of seven different plants, viz., *Azadirachta indica, Eucalyptus globulus, Tagetes minuta, Datura stramonium, Lantana camara, Ricinus communis* and *Jatropha curcas,* as natural larvicides against third instar larvae of *A. gambiae.* Insecticidal susceptibility tests were carried out using WHO standard method and the mortality was observed after 24 and 48 h (h) of exposure. The experiment was conducted in complete randomized design in three replications. Data were collected on mortality of mosquito larvae in all the treatments and then subjected to statistical analysis using one-way ANOVA. Most of the tested extracts showed more than 50% mortality. *J. curcas* (100±0.00%) and *R. communis* (99.44±0.56) gave significantly higher larval mortalities at 1000 ppm concentration

after 48 h of exposure. The experiment also showed that ethanol extracts gave higher larval mortality than hot water extracts and the efficiency of the extracts increased with an increase in the exposure period of the larvae.

Villanueva *et al.,* (2008) evaluated the larvicidal effect of aqueous extracts of 14 medicinal plants at 0.05% (weight: volume) against *Aedes aegypti* (L.) in Mexico. Bioassays were conducted with early fourth instars submerged in plant infusions to ingest the potential insecticide compounds. A preliminary bioassay for all plants showed that the highest mortality occurred in extracts from crushed and whole plants. The mean mortality for *Solanum nigrescens Martens & Galeotti, Operculina pteripes (G.Don) O'Donell* and *Phoradendron tamaulipensis Trel.* was 55, 17.5 and 5.8% respectively. Then, monthly bioassays with fresh field-collected *S. nigrescens* were conducted to evaluate seasonal variation in larvicidal activity with different plant parts. Only extracts of crushed (83-100% mortality) and entire (88 – 98% mortality) root were lethal to *A. aegypti* larvae. Monthly average mortality was 91.6 and 93.3% for crushed and whole root extracts of *S. nigrescens.*

2.5 LARVICIDAL PROPERTIES OF PLANTS EXTRACTS AND MOSQUITO LARVA

Many control strategies for mosquitoes have been suggested since the ancient times. Among the various control measures, viz., mechanical control by source of reduction (Mazzarri and Georghiou, 1995); biological control, using endopathogenic bacteria, *Bacillus thuringiensis* (Seleena *et al.,* 1995 and Mulla *et al.,*1999; Tabashnik *et al.,* 1994); larivorous fish (Gluckman and Hartney, 2000) as well as predatory arthropods (Bohidar and Mohapatra, 2000) and chemical control (Laird and Miles, 1983). The chemical insecticides, including organophosphates, organochlorines and pyrethroids are being utilized for the control of vector and mosquito

populations (Govindarajan *et al.,* 2013). Repeated use of chemical insecticide resulted in several problems such as environmental hazards, elimination of natural enemies, toxic residues in food, and also produced insecticidal resistance in major vector species (Macedo *et al.,* 1997). These and other pitfalls have compelled scientists to advocate for a refocus on botanicals, in Integrated Mosquito Management (IMM) protocols.

Natural products are generally preferred because of the innate biodegradability. More than 2000 plant species have been known to produce chemical factors and metabolites of value in the pest control programmes (Ahmed *et al.,* 1984) and among these plants, products of some 344 species have been reported to have a variety of activities against mosquitoes (Sukumar *et al.,*1991). The phytochemicals derived from plant sources possess a complex of chemicals with unique biological activity. The phytochemicals derived from plant resources can act as larvicidal, ovicidal, oviposition deterrence, growth and reproduction inhibitors, repellents, growth regulation, fecundity suppression, male sterility and smoke toxicity (Elimam *et al.,* 2009). Some of the plant leaves extracts are tested for their diverse insecticidal properties on the medically important mosquitoes: methanolic extract of *Derris elliptica* (Prempree and Sukhapanth,1990); aqueous extract of *Senna didymobotrya* (Ojewole *et al.,* 2000); aqueous extract of *Solanum nigrum* (Singh *et al.,* 2001); acetone extract of Solanum trilobatum (Rajkumar and Jebanesan, 2004); aqueous extract of *Gymnema sylvestre* and *Eclipta prostrate* (Khanna and Kannabiran, 2007); methanol, benzene and acetone extracts of *Cassia fistula* (Govindarajan, 2009); petroleum ether extract of *Azadirachta indica, Ocimum gratissimum* and *Hyptis suaveolens* (Okigbo *et al.,* 2010); aqueous and chloroform extracts of *Leucas aspera* (Ramanibai *et al.,* 2011); ethanolic extract *of Datura stramonium* (Swathi *et al.,* 2012); aqueous extract of *Spathodea campanulata* (Saranya *et al.,* 2013); methanolic extract of *Spathodea campanulata* (Karthika Devi *et al.,*

2013); acetone extract of *Spathodea campanulata* (Pravin *et al.,* 2014); aqueous extract of *Pithecellobium dulce* (Mahendran et al., 2015); acetone extract of *Spathodea campanulata* (Pravin *et al.,* 2015); aqueous extract of *Tecoma stans* (Navaneethan *et al*., 2016); ethanolic extracts of *Callistemon citrinus* (Palanikumar *et al.,* 2017); ethanolic extract of *Calotropis procera* (Mashlawi and Ali, 2017); methanolic leaf extract of *Senna alata* (Karthiyani *et al.,* 2018); methanolic extract of *Plectranthus barbatus* (Lawi *et al.,* 2018); aqueous and ethanolic extracts of *Carica papaya* and Cereus pterogonus (Sebastian *et al.,* 2018); methanolic extracts of *Seasamum indicum, Pungamia pinnata* and *Croton bonplandianum* (Dhanasekarn *et al.,* 2018) petroleum ether, ethyl acetate and aqueous extract of *Lantana indica* (Rathnasagar and Thiyagaraj, 2018); aqueous extract *of Cinnamomum tamala, Aloe vera, Datura alba, Allium sativum, Allium cepa, Zingiber officinale* and *Ocimum basilicum* (Iqbal *et al.,* 2018).

Schneider *et al.* (2017) reported the high potential of neem oil to control pupae and adults of *D. saccharalis* present in sugarcane. The study conducted by Lin *et al.* (2016) provided insight into the gene expression of *Monochamus alternatus* (vector of the destructive forest pest pinewood nematode) at the transcriptional level when subjected to azadirachtin, an active compound of neem, confirming its potential against the pest. This enhances the value of azadirachtin as a potential insecticide of natural origin. Besides, the neem extract is considered a growth regulator insecticide for the control of the lesser mealworm beetle *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) (Zorzetti *et al.,* 2015). In other applications, Forim *et al.* (2013) developed a method to prepare nanoparticles loaded with neem (*A. indica*) extracts, which presented a promissory larvicidal activity against *Plutella xylostella* with 100% larval mortality.

Pravin and Mohanraj, (2019) evaluated the qualitative phytochemical and GC-MS analysis, larvicidal and ovicidal properties of *Spathodea campanulata* hexane leaf extract against *Aedes*

aegypti. Bioassay test are carried out for testing the efficacy of hexane leaf extract of *S. campanulata* on *A. aegypti* at different stages of development viz I, II, III and IV instars and pupae. Instructions of WHO (1960) as detailed by Pampana (1963) for conducting bioassay experiment with mosquito larvae were carefully followed. Effect of hexane leaf extract of *S. campanulata* on the hatchability of *Ae.aegypti* eggs were determined and hatching rate was calculated on the basis of non-hatchability of eggs (Sahgal and Pillai, 1993). The qualitative phytochemical analysis revealed the presence of different phytochemicals such as carbohydrates, tannins, flavonoids, alkaloids, terpenoids, phenols, coumarins, and phytosteroids. Considerably low LC50, LC90/24, 48 hours' values of hexane leaf extract *S. campanulata* against different instar (I, II, III, IV and pupae) stages of *Ae. aegypti* obtained from this study proved the larvicidal, and ovicidal property of the plant. Young larvae were found to be relatively more susceptible than the older ones. The hatchability of *Ae. aegypti* eggs was decreased when placed in media of hexane leaf extract. The reduction in percent hatch was inversely proportional to the concentration of hexane leaf extract used. As the plant of the present study is widely distributed, the commercial exploitation could provide an important step in the development of new plant based insecticide as one of the alternative to expensive and environmentally harmful chemical insecticides.

Egunjobi and Okoye, (2020) evaluated the ovicidal and larvicidal activities of ethanolic leaf extracts of *Duranta erecta, Tridax procumbens* and *Pennisetum purpureum* against *A. gambiae.* Phytochemical analysis of these plants revealed the presence of tannins, saponins, alkanoids, flavonoids, glycosides and anthroquinone. Ground dry leaves of each plant material were concentrated in 7 litres of 95% ethanol for 72 hours followed by filtration and evaporation. *D. erecta, T. procumbens* and *P. purpureum* yielded 617.2g, 598.3g and 552g of extracts

respectively. The WHO standard for mosquito bioassay was adopted and concentrations 40, 100, 140 and 200 parts per million (PPM) were tested against 20 eggs and 25 larvae using emersion method. The hatching rate and % larval mortality of the extracts were recorded in which a concentration dependent increase was observed. High ovicidal activity (low egg hatchability) was recorded in D. erecta (LC_{50} -10.037 PPM) followed by *P. purpureum* and *T. procumbens* with LC_{50} values of 17.380 and 39.198 respectively. The highest larvicidal activity was observed in *D. erecta* (LC_{50} -76.943 PPM) compared to *P. purpureum* and *T. procumbens* (LC50 - 213.410 PPM and 214.217 PPM). Evidently, *D. erecta* ethanolic leaf extracts showed the best efficacy in the control of *A. gambiae* in this study. *D. erecta* is an environmentally friendly alternative in reducing the use of chemicals for mosquito control.

Manzano *et al.,* (2020) evaluated the ethanolic extract of Azadirachta indica leaves as a larvicide against Aedes aegypti and to determine the main compounds present in it by GC-MS. In the assay, three concentrations of ethanolic extract were used (10 mg L-1, 20 mg L-1, and 50 mg L-1). This was performed thrice against a positive control (commercial larvicide: spores and endotoxic crystals of *Bacillus thuringiensis* var. *israelensis* Serotype H-14) and negative control (water). After 72 h of incubation, it was observed higher larval mortality (93%) in the ethanolic extract at a concentration of 50 mg L-1; the extracts at 10 mg L-1 and 20 mg L-1 shown larval mortality of 47% and 70%, respectively. The majority compound determined by the GC-MS analysis was phytol (14.4% area). The results obtained in this study demonstrated the larvicidal potential of the ethanolic extract of *A. indica* against larvae of *A. aegypti.*

Oluah and Ezeabiakwa, (2011) Evaluated the larvicidal activity of leaf extract of *Lantana camara* (Family: Verbenaceae) against the *Aedes aegypti* Mosquito. Aqueous and ethanolic leaf extracts of *Lantana camara* were prepared and four different concentrations (0.3, 0.6, 0.9 and 1.2

g/ l) of both extracts were tested for larvicidal activity against the fourth instar larvae of *Aedes aegypti* for 24 hours in the laboratory. The 24-h percentage mortality rates ranged from 91.66 to 96.66 % in larvae treated with 0.3 to 1.2 g/L ethanolic *L. camara* leaf extract, respectively. The 24-h lethal time (LT50) and lethal concentration (LC50) were 6.8 h and 0.48 g/l, respectively. The aqueous extract did not result in more than 35 % larval mortality. The 24-h percentage mortality between the treatment groups were significantly different ($P < 0.001$) and also when compared with the control ($P < 0.05$). The result showed that the ethanolic extract was more lethal than the aqueous extract to *A. aegypti* larvae. The ethanolic extract also limited the pupation and the adult emergence that was inhibited by the ethanolic extract at 0.9 and 1.2g/L extract.

Aigbodion *et al.,* (2019) investigated the larvicidal activities of methanol leaf extracts of six tropical plants against *Anopheles gambiae* mosquitoes. 10 healthy laboratory stabilized larvae were treated with extracts of *Ocimum gratissimum, Chromolaena odorata, Terminalia catappa, Carica papaya, Vernonia amygdalina* and *Cymbopogon citratus* with different concentrations (0, 200, 400, 600, 800 and 1000 ppm) for 24, 48 and 72 hours after which the percentage mortality was calculated. All extracts tested were seen to possess moderate to good larvicidal effect against *An. gambiae* larvae in a concentration dependent manner with the highest mortality observed in *O. gratissimum* with 100%, *Cy. citratus* with 93%, *Ca. papaya* and *V. amygdalina* with 83%, *T. catappa* with 73% and the least being *Ch. odorata* with 63%at the end of the exposure period of 72 hours. These results showed that these plant extracts may be used as alternative insecticides against *An. gambiae* mosquitoes, with a further study on their phytochemicals, characterization and synergistic activities and their adaptability to field assay highly recommended.

Mahyoub *et al.,* (2014) studied the biological effects of various concentrations of *Melia azedarach, Rhazya stricta, Jatropha curcas, Artemisia herba alba, Calotropis procera, Matricharia chamomella* and *Diflubenzuron* were assayed on an *Aedes aegypti* (L.) test population under controlled laboratory conditions. Concentration levels of responses were evaluated. Characteristics such as IC_{50} and IC_{90} the susceptibility of immature stages to these plant extracts and insect growth regulator and their accumulation effects were studied. The percentage mortality of the fourth instar of *Ae. aegypti* larvae increased significantly with latex concentrations, indicating a direct relationship between the concentration and different effects. The larval mortalities ranged between low or moderate. According the mode of action of different plant extracts and Diflubenzuron did not appear to give high percentage of mortality against larval stages, although in most cases a clearly delayed inhibition of adult emergence was noted. The survival pupae percentage that produced from treated with different concentrations indicated that increased significantly of pupal survival due to decreasing the concentrations. There were significantly larval mortality and inhibition adult emergency percent in the treated groups compared to the control group. The characteristics investigated here indicate that this plant extracts and insect growth regulators are effective alternatives for controlling the dengue vector.

Bekele *et al.,* (2014) studied the larvicidal and adulticidal effects of extracts from some indigenous plants against the malaria vector, *Anopheles arabiensis* (Diptera: Culicidae) in Ethiopia. The mosquito Anopheles not only cause nuisance by their bites but also transmit deadly diseases like malaria in Sub-Saharan Africa, where most of the malaria deaths occur in the world. In Ethiopia, despite the use of native plants in traditional combat against mosquitoes, use in a modern way has remained scanty. The present study, being based on an initial ethno

botanical survey, carried out screening experiments on five indigenous ethno botanical species (*Aloe pirottae Berger, Aloaceae; Acokanthera schimperi (A.DC) Schweinf, Appocynaceae; Brassica nigra L. Koch, Brassicaceae; Oreosyce africana Hook.f., Cucurbitaceae and Piper capense L.f., Piperaceae*). The larvicidal activity of 80% methanol extracts of the first two plant species against the fourth instars of *Anopheles arabiensis* Patton, and adulticidal activity with the same solvent extracts of the latter four species against *Anopheles arabiensis* adults, gave positive results upon evaluation under laboratory condition. The 80% methanol extract of the gel of *A. pirottae* had more activity within 24 hours on the larvae than the leaf extract of *Acokanthera schimperi*. The highest (100%) mortality in the fourth instars occurred on treatment with 160 ppm extract of *Aloe pirottae* and 480 ppm extract of *Acokanthera schimperi*. The maximum adult mortality was detected in the leaf extract of *Oreosyce africana* (LC_{50} 18.74 and LC_{90} 39.66 ppm) followed by fruit extract of *Piper capense* (LC_{50} 24.30 and LC_{90} 46.32 ppm), while no mortality was noticed in the control groups. Phytochemical screening of the methanol extracts of the leaves of *Oreosyce africana* and the fruits of *Piper capense* had key secondary metabolites (alkaloids, saponins, flavonoids, cardiac glycosides), further corroborating their adulticidal properties. These findings announce the first evidence that Aloe pirottae is a promising mosquito larvicide while Oreosyce africana and Piper capense carry huge potentials as mosquito adulticides contributing to integrated malaria control through proper mosquito management.

Rotimi *et al.,* (2011) studied the Bioefficacy of Extracts of some Indigenous Nigerian Plants on the developmental stages of mosquito (*Anopheles gambiae*). The bioactivity of hexane extract from the nuts of *Anacardium occidentale* (Linnaeus), ethanol extracts from the bark of *Myrianthus arboreus* (*P. Beauv*) and fruits of *Xylopia aethiopica* (Dunal), were studied at five concentration levels (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) against the larvae, pupae and adults of

Anopheles gambiae (Giles). Results indicated that *X. aethiopica* caused significantly ($P < 0.05$) higher mortality of larvae, pupae and adult mosquitoes than other plant extracts tested. It caused 100%, 57.50% and 92.50% larva, pupa and adult mortality, respectively at 0.5% concentration. Also, based on the lethal concentration average (LC_{50}) results, *X. aethiopica* was the most effective, with LC_{50} values of 0.23, 0.40 and 0.29 µg/ml on the larvae, pupae and adults *An. gambiae,* respectively, followed by *A. occidentale* (LC_{50} 0.28, 0.45 and 0.34 µg/ml), then *M. arboreus* (LC_{50} 0.32, 0.64 and 0.36 µg/ml). The results of our findings were discussed in line with use of biorationals as an affordable, readily accessible, and environmentally friendly alternative means of reducing malaria disease in Nigeria, by controlling *An. gambiae* mosquito, a major vector of malaria pathogen.

Kasim *et al.,* (2019) designed a study to determine the larvicidal effect of aqueous extract of garlic against the 4th instars of *Culex* and *Anopheles* mosquito larvae. *Anopheles* and *culex* mosquito larvae were obtained using a deeper from stagnant water in the fadama at kofar Kade along illela road, Sokoto and taken to the Physiology laboratory of biological sciences, Usmanu Danfodiyo University Sokoto for further analysis. Fresh samples of garlic were obtained from central market, Sokoto state and were taken to the physiology laboratory for processing. The concentration of the extract used was 0.5mg/ml, 2.0mg/ml and 3.0mg/ml were obtained by weighing 0.5mg, 2.0mg, and 3.0mg in 10ml of water. Mortality of *Culex* and *Anopheles* depends on the garlic extract and increase with time of exposure and concentration of the extract. 3.0mg/ml recorded the highest mortality rate of 3 hours of exposure for both *Culex* and *Anopheles* at a mean of 10.00 each while 0.5mg/ml recorded minimum mortality after 1 hour of exposure for both anopheles and *Culex* with mean of 2.33 and 3.67 respectively. The study

demonstrated the potency of garlic (*Allium sativum*) in managing the larvae and thus contributes as an affordable way to control *Anopheles* and *Culex* larvae of mosquito.

2.6 LARVICIDAL EFFICACY OF INDIGENOUS PLANT EXTRACTS AGAINST CULEX SPECIES

Danga *et al.,* (2014) assessed the phytochemicals and larvicidal activity of different solvent leaf extracts of *Plectranthus glandulosus* against three major vector mosquitoes, viz. *Anopheles gambiae, Aedes aegypti* and *Culex species.* Twenty-five early fourth instar larvae of each mosquito species were exposed to various concentrations ranging from 1251000 ppm of methanol crude extract (MCE), hexane (HF), chloroform (CF), ethyl acetate (EAF) and methanol fractions (MF), from 250-2000 ppm of water extract (AE) and 2000 ppm of DDVP. The WHO standard protocol was followed. The larval mortality was observed 24 h post-exposure. The LC50 and LC90 values were determined by probit analysis. The qualitative phytochemical analysis revealed the presence of Alkaloids, Terpenoids, Steroids, Saponins, Tannins and Phenolic Compounds, Lipids, Fats and Fixed Oils. Among all solvent extracts and fractions tested, the maximum efficacy was observed in HF against all target mosquito species with LC50 values of 17.11, 89.08 and 610.40 ppm against *A. gambiae, A. aegypti and C. species,* respectively. MCE, CF and EAF against *A. gambiae* were also effective with LC50 values of 167.85, 201.50 and 76.21 ppm, respectively. The results of the leaf extracts and fractions of *P. glandulosus* are promising as good mosquito larvicides against *A. gambiae, A. aegypti* and *C.* species.

Modise *et al.,* (2016) on a similar study, evaluated the larvicidal, pupicidal and insecticidal activities of *Cosmos bipinnatus, Foenuculum vulgare* and *Tagetes minuta* leaf extracts against *Culex species* mosquitoes. The leaves of the plants were extracted with distilled water, ethanol

(95 %), and hexane and the extracts screened for their phytochemical profile. While larvicidal and pupicidal activities were assayed at concentrations ranging from 0.1 - 10 mg/mL, insecticidal property was tested at varying amounts (0.25 - 2 g) of the plant sample. The respective larval mortality was thereafter evaluated using Probit analysis. Saponins, terpenoids, flavonoids and steroids were detected in the plant extracts. The ethanol extracts of *F. vulgare, T. minuta* and *C. bipinnatus* exhibited larvicidal activity half-maximal lethal concentration (LC_{50}) of 0.10, 1.17 and 1.18 mg/mL, followed by hexane extracts with LC_{50} value of 1.03, 1.01 and 1.27 mg/mL, respectively, against the larvae of *C.* species mosquito. Hexane extracts displayed pupicidal activity with LC_{50} of 1.07, 1.12 and 1.16 mg/mL against *F. vulgare, T. minuta* and *C. bipinnatus,* respectively, while the ethanol extracts of *T. minuta, C. bipinnatus* and *F. vulgare* displayed pupicidal activity at LC_{50} of 1.11, 1.14 and 1.31 mg/mL respectively, against pupa of *C. species* mosquito. The aqueous extracts had no ($p > 0.05$) lethal effects on both larvae and pupa of *Culex* species at all evaluated concentrations. *F. vulgare* had the highest ($p < 0.05$) half-maximal knock-down effect (KD_{50} = 7.52 min-1), followed by *T. minuta* (KD_{50} = 8.64 min-1) on adult *C. species* mosquitoes after 6 h of exposure. *F. vulgare* and *T. minuta* killed all evaluated mosquito adults within 12 h with LD99 = 0.25 g/air, while the leaves of *C. bipinnatus* had no ($p > 0.05$) knock-down or lethal effects on the adult mosquito. *C. bipinnatus, F. vulgare and T. minuta possess* larvicidal and pupicidal properties against *C. species, whereas only F. vulgare and T. minuta* displayed insecticidal properties. Consequent upon these findings, all the plants can be considered naturally potent larvicidal and pupicidal agents against *C.* species.

Iqual *et al.,* (2019) focused on the insecticidal potential of easily available local botanicals using a simple but effective method. Seven indigenous plants (*Cinnamomum tamala* (taiz pat), *Aloe vera* (aloe vera), *Datura alba* (datura), *Allium sativum* (garlic), *Allium cepa* (onion), *Zingiber*

officinale (ginger), and *Ocimum basilicum* (niazbo/basil) were tested for their larvicidal efficacy against *Culex* species under laboratory conditions. The evaluation of a series of five concentrations (1%, 2%, 3%, 4%, and 5%) of aqueous plant extracts against the 4th instar larvae revealed convincing larval mortality effects at 24 and 48 hr. after exposure. Larval mortality showed a significant concentration-dependent correlation. No mortality was observed in the control. The LC50 values demonstrated garlic as the most effective (1.37%), followed by taiz pat (1.48%) and aloe vera (1.96%), at 24 h. Moreover, the LC50 at 48 h showed high efficiency by aloe vera (0.37%), followed by garlic (0.55%) and taiz pat (0.98%). The sequence of LC50 values for the other plants were onion (2.20%) < datura (2.49%) < niazbo (5.32%) < ginger (7.48%) after 24 hr and datura (1.13%) < niazbo (1.17%) < onion (1.24%) < ginger (2.43%) after 48 hr. Taken together, the aqueous extracts of all plants exhibited potential efficacy against *C.* species larvae and could be considered as potent natural larvicidal agents. These plants may be recommended for use in mosquito management programs as potential alternatives to synthetic insecticides. The simple aqueous extraction method is easy and inexpensive and can be used at the home level for mosquito management.

Otabor *et al.,* (2019) assessed the larvicidal efficacy of the methanolic extract of Cymbopogon citratus, Ocimum gratissimum and Vernonia amygdalina against the third instar larvae of Culex *species*. Qualitative analysis of the plants revealed that alkaloids, flavonoids, saponins, steroids, tannins, terpenoids and glycosides were present in all three plant extracts. Phlobatannins was present in trace amounts in O. gratissimum and absent in C. citratus and V. amygdalina. Larvicidal activities of the leaf extracts were studied on laboratory reared larvae of *C.* species at a concentration range of 250 ppm to 1000 ppm. The percentage mortality was calculated and LC50, LC90 values were obtained from probit analysis using SPSS version 16.0 at 95%

confidence limit (CL). Result of this study indicated that the percentage mortality of O. gratissimum extract was dose dependent with 250 and 1000 ppm having the percentage mortality of 18.33 and 43.3% respectively after 72 hrs. The percentage mortality in C. citratus extract after 72 hrs was 66.67% at 1000ppm concentration whereas at 750 ppm mortality was 8.33%. The percentage mortality for V. amygdalina increased from 250 to 750 ppm but decreased at 1000ppm with 750 ppm having a mortality of 63.33% and 1000ppm having a percentage mortality of 56.6% after 72 hrs. The LC50 and LC90 values of the methanolic leaf extract obtained after 72 hrs was 1008.19 and 1930.992 ppm for C. citratus, 1148.47 and 2210.727 ppm for O. gratissimum and 754.712 and 1548.499 ppm for V. amygdalina respectively. The methanolic extract of V. amygdalina exhibited a higher degree of potency when compared with the methanolic extract of C. citratus and O. gratissimum with a low LC50 value of 758.403ppm, 758.03 ppm and 754. 712 ppm at 24, 48 and 72 hrs respectively. In summary, this study reports the larvicidal effects *of C. citratus, O. gratissimum* and *V. amygdalina against Culex species* larvae which can serve as an alternative to synthetic pesticides in Nigeria.

CHAPTER THREE

MATERIALS AND METHOD

3.1. STUDY AREA

This study was conducted in the premises of University of Benin, Animal and Environmental Biology Department, Faculty of Life Sciences and the Department of Pharmacognosy, Faculty of Pharmacy.

3.2. PLANT COLLECTION, IDENTIFICATION AND PREPARATION

Fresh leaves and bark of *Chrysophyllum albidum* were collected from Ajakurama community, located within Longitudes 5°10.161'E and Latitudes 6°10.723 N, Ovia South west, Edo state. They were identified by a botanist at the department of plant biology and biotechnology, University of Benin. Collected leaves and bark of the plant was rinsed with tap water and sun dried for some days and then pulverized to powder level, using the British milling machine at the department of Pharmacognosy.

3.3. PREPARATION OF STOCK SOLUTION

Standard WHO (2005) procedure was adopted in this study with slight modifications. 2% stock solution was used for each extract of *Chrysophyllum albidum* leaves and barks. This was prepared by dissolving 2g of solid extract in 100ml of water.

3.4. MOSQUITO COLLECTION AND REARING

Samples of *Culex* egg raft were collected from breeding sites around University of Benin, Ugbowo campus. The egg raft was introduced to plastic bowls in the lab and yeast was added to it, then the eggs emerged as larvae after some days. Colony was raised in the Department of Animal and Environmental Biology. The second and third instar Larvae was used for the experiment and few was left in the plastic bowls and feed with yeast. Pupae were transferred into a 0.4m × 0.4m× 0.4m (L × B×H) mosquito rearing cage where they emerged as adults which was now used for the identification of the species.

3.5. LARVAL BIOASSAY

The concentrations used for bioassay were 500ppm, 750ppm and 1000ppm respectively. To perform the larvicidal bioassay, 5.0, 7.5, and 10 ml of stock solution were each diluted to 100 ml in separate plastic containers to makeup 500, 750 and 1000 ppm test concentrations respectively. A test solution without any plant extract was used as control (0ppm). 10 second and third instar larval stages of Culex sp. mosquitoes were introduced into each test bowls with the various concentrations for72 hrs. Experimental plastic bowls were replicated thrice and maintained at a room temperature of 31± 0.2 °C and a relative humidity of 53 ± 0.7%.

Dead and moribund larvae were counted at an interval of 24, 48 and 72hrs respectively for all treatments. Larvae were considered dead when they remain still at the bottom of the test

containers and do not come up to the surface. No food material was added to the control or test solution.

3.6. QUALITATIVE PHYTOCHEMICAL SCREENING

The phytochemical constituents of the plants were analyzed according to reports from Evans (1989) and Sofomare (1993). The extracts were screened for the presence of Alkaloids, Tannins, Saponin, Flavonoids, Steroids and Carbohydrates.

3.6.1 TEST FOR CARBOHYDRATES:

Add 2 drops of ethanoic extract of the sample, 2 drops of 10% alcoholic oil of naphthol and 2 drops of concentrated H_2SO_4 into a test tube inclined at 45^o. A brown red coloration at the interface between the acid and the extract was observed indicating the presence of carbohydrate.

3.6.2. TEST FOR SAPONIN:

Two drops of the filtrated plant extract and 4ml of H_20 was heated, filtered and left to cool. A persistent frothing was observed indicating the presence of saponin.

3.6.3. TEST FOR TANNINS:

Two drops of ethanoic extract, 5 ml of ferric ammonium citrate and 5g of sodium acetate was added to attest tube boiled and left to cool. A black to bluish precipitate was observed indicating the presence of Tannin

3.6.4 TEST FOR FLAVONOIDS:

Two drops of the ethanoic extract, 1 drop of NaOH and I drop of diluted HCL was added to a test tube. A colourless precipitate was observed indicating the absence of flavonoids.

3.6.5 TEST FOR REDUCING SUGAR:

Two drops of the ethanoic extract and Fehling's solution (AH_3) was boiled and left to cool. A persistent blue solution was observed indicating the presence of reducing sugars.

3.6.6. TEST FOR ALKALOIDS:

Two drops of the ethanoic extract of the sample, 1 drop of Dragendorff reagent, Mayer reagent and picric acid. A cream brown precipitate was observed indicating the presence of alkaloids.

3.7 PERCENTAGE MEAN MORTALITY FOR EACH CONCENTRATION

$$\%\text{Mortality} = \frac{\text{Number of dead larvae}}{\text{Total of larvae introduced}}$$

3.8. DATA ANALYSIS

The percentage mortality was calculated. The mortality effect was analyzed using one-way factorial Analysis of Variance (ANOVA) on Statistical Package for Social Scientists (SPSS). The Duncan's Multiple Range test (DMR) was employed to further analyse the significant difference among the various test treatments. Significance in comparison was set at $P<0.05$. Larval mortality data obtained was subjected to probit analysis on SPSS to determine the lethal concentrations (LC50 and LC90) of each plant extract against the *Culex* sp. larvae at 95% confidence limits.

CHAPTER FOUR

RESULTS

4.1 PHYTOCHEMICAL CONTENT

The phytochemicals screening of ethanoic extract of *C. albidum* from both the leaves and stem bark revealed the presence of carbohydrate, saponins, tannins, phenol, steroid and alkaloids in *C. albidum* and the absence of phenol in leaves (Table 1).

Table 1: Qualitative phytochemical constituents of ethanoic extract of *C. albidum*

Plant parts	**Carbohydrates**	**Phenol**	**Steroid**	**Saponin**	**Tannins**	**Flavonoids**	**Alkaloids**
Leaves	+	-	+	+	+	+	+
Stem bark	+	+	+	++	++	+	+

Key: +++ obviously present, ++ slightly present, - absent

4.2 EFFECT OF TIME AND CONCENTRATION ON LARVAL MORTALITY

4.2.1 EFFECT OF CONCENTRATION OF C. ALBIDUM BARK AGAINST CULEX SP.

The mortality of *Culex* larvae exposed at 24 hours to the test concentration of *C. albidum* bark showed no significant difference ($p>0.05$). However larval mortality resulted from continuous exposure to the test concentration between 48-72 hours of exposure had significant difference. ($p<0.05$) (Table 2). Highest mortality was observed at 1000 ppm concentration at 72 hours of *Chrysophyllum albidum* (Table 2). Highest mortalities per exposure time were observed in larvae exposed to highest test concentration of 1000 ppm (24 h = 3.3%; 48 h = 10.0%; 72 h = 13%)

Lethal concentration of *Chrysophyllum albidum* bark against *Culex* larvae at various time interval were determined. At 24 hours the LC_{50} and LC_{90} were 324752.349ppm and 7872507.917ppm. At 48 hours the LC_{50} and LC_{90} were 13736.074 ppm and 149795.053ppm. At 72 hours LC_{50} and LC_{90} were 6332.262 ppm and 58278.321 respectively. (Table 3).

4.2.2 Effect of concentration of *Chrysophyllum albidum* leave against *Culex* sp.

There was no significance difference ($P>0.05$) in the mortality of *Culex* larvae exposed at 48 hours to the test concentration of *C. albidum* leaf. However, the mortality of *Culex* larvae exposed at 24 and 72 hours to the test concentration of *C. albidum* bark showed a significant difference ($p<0.05$). Highest mortality was observed at 1000 ppm concentration of *C. albidum* for 72 hours (Table 4). Highest mortalities per exposure time were observed in larvae exposed to highest test concentration of 1000 ppm (24 h = 10%; 48 h = 16.7 %; 72 h = 23.3%)

Lethal concentration of *Chrysophyllum albidum* leaf against *Culex* larvae at various time interval were determined. At 24 hours the LC_{50} and LC_{90} were 17200.306 ppm and 197975.707ppm. At

48 hours the LC_{50} and LC_{90} were 3013.400 ppm and 11200.179 ppm. At 72 hours LC_{50} and LC_{90} were 4672.394 ppm and 46866.555 respectively. (Table 5).

4.2.3. EFFECT OF CONCENTRATION OF CHRYSOPHYLLUM ALBIDUM BARK AND LEAF AGAINST CULEX SP.

The mortality of *Culex* larvae exposed at 24, 48 and 72 hours to the test concentration of *Chrysophyllum albidum* bark and leave showed no significant difference ($p<0.05$). Highest mortality was observed at 1000 ppm concentration of *Chrysophyllum albidum* for 72 hours (Table 6). Highest mortalities per exposure time were observed in larvae exposed to highest test concentration of 1000 ppm (24 h = 6.7%; 48 h = 13.0 %; 72 h = 23.3%)

Lethal concentration of *Chrysophyllum albidum* leaf against *Culex* larvae at various time interval were determined. At 24 hours the LC_{50} and LC_{90} were 58689.904ppm and 916008.897 ppm. At 48 hours the LC_{50} and LC_{90} were 8676.959 ppm and 84888.706ppm. At 72 hours LC_{50} and LC_{90} were 4265.617ppm and 42292.050 respectively. (Table 7).

Table 2: Effect of concentration of *Chrysophyllum albidum* bark against *Culex sp.*

			Mean ± SD (Percentage mortality)		
	Conc. (ppm)	n	24 hours	48 hours	72 hours
	0	3	0.00±0.00 (0.00)	0.00±0.00(0.00)	0.00±0.00(0.00)
	500	3	0.00 ±0.00 (0.00)	0.00±0.00 [b](0.00)	0.67±0.00(6.7)
	750	3	0.00±0.00 (0.00)	0.67±0.00 [a](6.7)	1.33±0.57 [a](13)
	1000	3	0.33±0.57 (3.3)	1.00 ±0.00 [a](10.0)	1.33±0.57 [a](13)
	F-value		1.00	7.00	3.0
	P-value		0.422	0.027	0.125

P<0.05 – Significantly difference P> 0.05 Not Significantly difference

Table 3: Lethal concentration of *Chrysophyllum albidum* extract against *Culex* Larvae.

		Lethal concentration (ppm)
24 hours	LC_{50}	324752.349
	LC_{90}	7872507.917
48 hours	LC_{50}	13736.074
	LC_{90}	149795.053
72 hours	LC_{50}	6332.262
	LC_{90}	58278.321

Table 4: Effect of concentration of *Chrysophyllum albidum* leave against *Culex sp.*

Concentration (ppm)	n	Mean ± SD (Percentage mortality)		
		24 hours	48 hours	72 hours
0	3	0.00±0.00(0.00)	0.00±0.00(0.00)	0.00±0.00(0.00)
500	3	0.00 ±0.00[a](0.00)	0.33 ±0.58[b](3.3)	1.00±0.58(10.0)
750	3	0.33±0.58[b](3.3)	1.00±0.00 [ab](10.0)	1.33±0.00 b(13.0)
1000	3	1.00±0.00 [a](10.0)	1.67 ±0.58 [a](16.0)	2.33±0.58 [a](23.3)
F-value		7.00	6.00	6.500
P-value		0.027	0.37	0.031

P<0.05 – Significantly difference P> 0.05 Not Significantly difference

Table 5: Lethal concentration of *Chrysophyllum albidum* leave extract against *Culex* Larvae.

		Lethal concentration (ppm)
24 hours	LC_{50}	17200.306
	LC_{90}	197975.707
48 hours	LC_{50}	3013.400
	LC_{90}	11200.179
72 hours	LC_{50}	4672.394
	LC_{90}	46866.555

Table 6: Effect of concentration of *Chrysophyllum albidum* bark and leaf against *Culex* sp.

Concentration (ppm)	n	Mean ± SD (Percentage mortality)		
		24 hours	48 hours	72 hours
0	3	0.00±0.00[a](0.00)	0.00±0.00(0.00)	0.00±0.00(0.00)
500	3	0.00 ±0.00[a](0.00)	0.33 ±0.58[b](3.3)	1.33±0.58[a](0.00)
750	3	0.33±0.00[b](3.3)	1.00±0.00 [ab](10.0)	1.33[b]±0.00(13.3)
1000	3	0.67±0.58[a](6.7)	1.33±0.58 [a](13.0)	2.33±0.58 [a](23.3)
F-value		4.00	3.50	3.00
P-value		0.079	0.098	0.125

P<0.05 – Significantly difference P> 0.05 Not Significantly difference

Table 7: Lethal concentration of *Chrysophyllum albidum* extract of both bark and leaf against *Culex* Larvae.

		Lethal concentration (ppm)
24 hours	LC_{50}	58689.904
	LC_{90}	916008.897
48 hours	LC_{50}	8676.959
	LC_{90}	84888.706
72 hours	LC_{50}	4265.617
	LC_{90}	42292.050

CHAPTER FIVE

5.0 DISCUSSION

Exploring bioactive medicinal plants in vector management program is one of the eco-friendly approaches because they are easily biodegradable. Naturally plants are rich store houses for potential bioactive compounds which are gaining appreciation in recent times among the scientific communities. According to Ekpoma *et al.* (2022) crude extracts of the plants may have mixtures of active compounds which act synergistically and their overall bioactivity was also greater than individual compounds.

5.1 Phytochemical Analysis of Plant Extract (*Chrysophyllum albidum*)

The effectiveness of this plant could be attributed to the presence of phytochemical compounds that act as insecticides (Abayomi,1993). *Chrysophyllum albidum* leaves and stem bark have furnished carbohydrates, saponin, tannins, phenol, steroids, flavonoids and alkalodis however phenol was absent in the leaf. These compounds were previously reported to have mosquito larvicidal activity (Farooq *et al.,*1999).

These secondary metabolites were previously reported by Oladimeji *et al.,* (2012), Adefolalu *et al.,* (2015), Ubulom *et al.,* (2019); Otabor *et al*., (2019) and Funmilayo, Ikem (2020). These metabolites when present in plants can exhibit high larvicidal potency which was observed in leaf and stem bark of *Chrysophyllum albidum* extract.

For this study, the larval mortality may be attributed to the phytochemical compounds detected in the extracts which could have exerted their effect on the larvae either individually or in synergy. Thaswin *et al.* (2019) observed the presence of tannin, saponin, and flavonoid compounds from the water-ethanol fractions of ketapang leaf extract, which agrees with

the findings of this study, but flavonoid was not detected in *C. albidum* seed extract. Tannins impede an insect's capability to further breakdown nutrients and utilise protein by attaching themselves to vital proteins needed for development. Some known major toxicities of tannins include increased cytoplasmic vacuolation, lack of cytoplasmic restrictions, apical vesicle materialisation accompanied by the discharge of cytoplasmic constituents of the cell, augmented intercellular space, and detachment of cells from the basement membrane, which resemble the processes of encountering a toxic substance (Thaswin *et al.,* 2019). Saponins exerts membrane-permeabilising and haemolytic properties, attacking the cuticle membrane of the larvae, and disturbing the membrane, which leads to larval death. They result in elevated death rates and reduced diet intake thus initiating a decrease in weight, delayed growth, instabilities during growth, and reduced reproduction in insect pests. The mechanism of action results from saponins deterring their urge to consume food or brings about gastrointestinal complications owing to moulting flaws or its toxic properties on cells (Thaswin *et al*., 2019). Flavonoid compounds also possess promising larvicidal potential, which could be the reason of higher mortality as exhibited by *C. mucunoides* leaf extract. Otabor *et al.* (2019) recorded a moderate quantity of glycosides in *O. gratissimum*, *C. citratus,* and *V. amygdalina*, which was in contrast to the results of this study as glycosides were absent in the extracts of *C. mucunoides* leaves and *C. albidum* seeds. It is important to note that the discrepancy in phytochemical constituents can be dependent on the polarity of extraction solvents, extraction methods employed, geographical origin, plant age, and climatic conditions

Danga *et al.,* (2014) showed that the solvent leaf extracts of *Plectranthus glandulosus* analysis revealed the presence of Alkaloids, Terpenoids, Steroids, Saponins, Tannins and Phenolic Compounds, Lipids, Fats and Fixed Oils and can be used as a good mosquito larvae agent. These

compounds may jointly (or) independently contribute to larvicidal activity against *A. aegypti.* The phytochemicals interfered with functioning of mitochondria (Usta *et al*., 2002) and primarily affect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae (Rey *et al*.,1999; David *et al.*, 2003). Tannins are toxic by blocking the digesting of foods causing growth disturbances (Lumowa and Nova, 2014), also causing a water-absorption disorder in the larvae, thus causing death to the larvae (Steyaningsih and Pasmiati,2015). Alkaloids are nitrogenous compounds that show insecticidal properties at low concentration and the mode of action on insect vectors varies with the structure of their molecules, but many are reported to affect acetylcholinesterase (AChE) or sodium channels as inhibition of acetylcholinesterase activity is responsible for terminating the nerve impulse transmission through synaptic pathway (Rattan, 2010; Liu *et al.,* 2012). Alkaloids work by constricting blood vessels and depressing autonomic nervous system activity thereby contributing to the insecticides effectiveness in killing the larvae of mosquitoes and disrupting the life cycle of the mosquito (Simon-Oke *et al.,* 2015).

5.2 LARVICIDAL EFFECT OF CHRYSOPHYLLUM ALBIDUM EXTRACT AGAINST CULEX LARVAE.

The *Chrysophyllum albidum* leaf extract was observed to exhibit higher mortality than the *Chrysophyllum albidum* stem bark extract against Culex larvae.

From this study, the least mortality was recorded at 24 hours (1.00) when exposed to 500 ppm of the test concentration of *Chrysophyllum albidum.* The highest mortality was recorded at 72 hours (3.00) when exposed to 1000 ppm of the test concentration of *Chrysophyllum albidum.* No mortality was recorded in the control. Total mortality was consistently positively correlated with plant extract concentrations and the duration of exposure. This was in agreement with the work

of Essam *et al.* (2005) who showed that the effect of *Callitris glaucophylla* extracts on the development of *Culex aegypti* was higher as the concentration in increases. The effects of these plant extracts on the targeted organisms have further confirmed the insecticidal potentials of some metabolic compounds produced by plants that will be environmentally friendly.

5.3 CONCLUSION

The findings of the present investigation revealed that the ethanoic leaf extract of *Chrysophyllum albidum* showed more larvicidal activity against *Culex* sp. compared to the bark. Increase in stock solution of ethanoic leaf extract of *Chrysophyllum albidum* offer promise in future mosquito control programs. Also a trial on the aqueous leaf extract of *Chrysophyllum albidum* should be considered.

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