

PHYTOCHEMICAL ANALYSIS AND LARVICIDAL ACTIVITY OF *ALOE VERA*

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CERTIFICATION

This is to certify that this research project titled “**Phytochemical Analysis and Larvicidal Activity of *Aloe Vera***” by “Abdulganiyu Oyindamola Faridat with admission number: 2010302161” has met the requirements for the award of Bachelor of Science Degree in Biology of Usmanu Danfodiyo University Sokoto and has approved for its contribution to knowledge.

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DEDICATION

I dedicated this research work to my beloved Parents: Alh. Abduganiyu Adeshola and Mrs. Abduganiyu Mujidat, for their love, support and prayers may Almighty Allah SWT rewards you abundantly.

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ABSTRACT

This study assessed phytochemical constituents and larvicidal activity of *aloe vera* leaf extract. Aloe vera, sometimes described as a “wonder plant”, is a short stemmed succulent shrub growing to 60-100cm (24-39 inch) tall and spreading by offsets. The leaves are thick and fleshy, green to grey-green with some varieties showing white flecks on the upper and lower stem surface (Yates, word 2013). Fresh leaves of *Aloe vera* were collected from cultivated sources within or near the university premises. The plant were authenticated by a taxonomist in the Department of Biological Sciences, UDUS. The collected leaves were thoroughly washed with clean water to remove dust and debris. The green outer rind were removed to extract the inner gel. The result indicates that *Aloe vera* leaves contain numerous secondary metabolites with potential bioactive and therapeutic effects. The high presence of saponins and glycosides suggests possible insecticidal and larvicidal properties, while flavonoids and tannins contribute to antioxidant and antimicrobial activities. The non-detection of anthraquinones implies their absence or very low concentration in the ethanolic extract. The study demonstrated that ethanolic leaf extract of *Aloe vera* contains several important phytochemical constituents, including saponins, glycosides, volatile oils, and flavonoids, which contribute to its significant larvicidal activity. The extract showed a concentration-dependent mortality effect on mosquito larvae, with the highest mortality observed at 250% concentration after 72 hours of exposure. The study recommended; Advanced studies should be carried out to isolate and characterize the specific bioactive compounds responsible for larvicidal activity in Aloe vera using chromatographic and spectroscopic techniques.

CHAPTER ONE

1.0

INTRODUCTION

1.1 Background to the study

Aloe vera, sometimes described as a “wonder plant”, is a short stemmed succulent shrub growing to 60-100cm (24-39 inch) tall and spreading by offsets. The leaves are thick and fleshy, green to grey-green with some varieties showing white flecks on the upper and lower stem surface (Yates, word 2013). The flowers are produced in summer on a spike up to 90cm (35 inch) tall, each flower being pendulous with a yellow tubular corolla 2-3cm (0.8 1.2 inch) long. The name Aloe is derived from the Arabic “Alloeh”, meaning shining bitter substances while Vera means “true”. Like other Aloe species, Aloe vera forms *arbuscular* mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil (Gong *et al.*, 2020). Some related Aloes occur naturally in North Africa (Bibsy *et al.*, 2017) but is widely cultivated throughout the world and largescale agricultural production is undertaken in Australia, Bangladesh, Cuba, the Dominican Republic, China, Mexico, India, Jamaica, Kenya, Tanzania and South African along with the united states of America to supply the Cosmetic industry with Aloe vera gel (United States Department of Health and Human Services, 2010).

Aloe vera produces two substances, gel and latex. Aloe gel is the clear, jelly- like Substances found in the inner part of the Aloe plant leaf. Aloe latex comes from the plant’s skin and is yellow in colour. Phytochemicals are plant chemicals that protect them against

bacteria and viruses. The action of phytochemicals varies by colour and type of food. They may act as antioxidants or nutrient protectors or prevent carcinogens (Hazra *et al.*, 2008). A fungus is any member of a large group of eukaryotic organisms that includes microorganisms such as yeasts and molds as well as the more familiar mushrooms. These organisms are classified as a kingdom, which is separate from plants, animals, protists and bacteria. The cell walls of fungal cells contain chitin unlike the cell walls of plants and some protists that contain cellulose and also different from the cell walls of bacteria.

Aloe vera is a perennial, drought-resistant plant belonging to the family *Asphodelaceae*. It contains a wide spectrum of secondary metabolites, such as anthraquinones, flavonoids, alkaloids, saponins, and tannins, which have demonstrated various biological activities including antibacterial, antifungal, and insecticidal effects (Yusuf *et al.*, 2021). The larvicidal potential of *A. vera* is increasingly being studied due to its eco-friendly nature and effectiveness against vector larvae, particularly species within the *Anopheles*, *Culex*, and *Aedes* genera (Kovendan *et al.*, 2012).

Phytochemical screening of *Aloe vera* gel and leaf extracts has revealed the presence of multiple bioactive compounds with potential insecticidal actions. These compounds may interfere with larval development or physiology, leading to mortality or impaired growth (Govindarajan & Rajeswary, 2015). Understanding the phytochemical profile of *A. vera*

and its larvicidal mechanism is essential for developing plant-based larvicides that are sustainable and less harmful to non-target species.

1.2 Statement of the Research Problem

Conventional chemical insecticides, while effective, have raised concerns regarding environmental pollution, non-target organism toxicity, and the development of insecticide-resistant mosquito populations (Kumar *et al.*, 2021). These issues underscore the urgent need for alternative, eco-friendly larvicidal agents derived from botanical sources. Aloe vera, a plant renowned for its medicinal and pharmacological properties, has demonstrated potential larvicidal activity attributed to its rich phytochemical constituents such as anthraquinones, saponins, and polysaccharides (El-Sayed *et al.*, 2023). Despite existing studies indicating Aloe vera's larvicidal efficacy, there remains a limited understanding of the specific phytochemicals responsible and their mechanisms of action. Additionally, standardized phytochemical profiles and systematic evaluation of larvicidal potency are lacking, which hinders the development of Aloe vera-based biopesticides.

1.3 Justification of the Study

Botanical plants like Aloe vera offer a promising solution due to their rich phytochemical composition and biodegradability, which minimize ecological impact (El-Sayed *et al.*, 2023). Moreover, Aloe vera is widely available, cost-effective, and has a long history of medicinal use, making it a viable candidate for developing sustainable vector control tools

(Reynolds, 2020). Scientific investigations have indicated that certain phytochemicals in *Aloe vera* possess larvicidal properties, but a detailed understanding of their specific constituents and mechanisms remains limited. Systematic phytochemical analysis coupled with bioassays is essential to identify active compounds and optimize their larvicidal efficacy (Khan *et al.*, 2023).

This research will contribute valuable knowledge towards harnessing *Aloe vera*'s potential as a natural larvicide, supporting integrated pest management strategies and reducing reliance on harmful synthetic chemicals. The exploration of *A. vera* as a larvicidal agent aligns with the global pursuit of environmentally friendly vector control methods. This plant is widely accessible, easy to cultivate, and renewable. Investigating its phytochemical content alongside larvicidal assays can provide an evidence-based foundation for formulating plant-derived larvicides. Additionally, characterizing its phytochemicals may contribute to the development of novel bioinsecticides, thus reducing dependence on synthetic chemicals and mitigating issues of resistance (Bhatt *et al.*, 2020).

1.4 Objectives of the Study

The main objective of this study is to evaluate the phytochemical constituents and larvicidal activity of *Aloe vera* leaf extract. The specific objectives of the study are:

- i. To identify the phytochemical constituents, present in *Aloe vera* leaf extract.

- ii. To assess the larvicidal efficacy of *Aloe vera* extract against selected mosquito larvae.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical Description and Distribution of *Aloe vera*

Aloe vera (L.) *Burm.f.*, a member of the *Asphodelaceae* family, is a succulent plant species widely known for its medicinal and therapeutic applications. It is a short-stemmed shrub that typically grows up to 60–100 cm in height and has thick, fleshy, lanceolate leaves arranged in rosettes. The leaves are green to grey-green, sometimes with white flecks on their surfaces, and are serrated along the margins with small white teeth (Reynolds & Dweck, 2019).

This plant is native to the arid and semi-arid regions of the Arabian Peninsula but is now cultivated globally due to its wide adaptability and commercial value. It thrives in warm climates and is commonly found in Africa, Asia, India, the Mediterranean, and parts of the Americas (Eshun & He, 2004). In Nigeria and other parts of West Africa, *Aloe vera* is grown domestically and used in traditional medicine, skincare, and, increasingly, in biological pest control strategies.

The plant consists of three main layers:

1. **The rind (pericyclic cells)** – the outer thick layer containing vascular bundles.
2. **The latex (yellow sap)** – a bitter exudate found beneath the rind, rich in anthraquinones.

3. **The gel (inner parenchyma)** – a clear, mucilaginous substance containing water, polysaccharides, vitamins, and bioactive compounds (Yusuf *et al.*, 2021).

Table 2.1: Taxonomic Classification of *Aloe vera*

Taxonomic Rank	Classification
Kingdom	Plantae
Subkingdom	<i>Tracheobionta</i> (Vascular plants)
Superdivision	<i>Spermatophyta</i> (Seed plants)
Division	<i>Magnoliophyta</i> (Flowering plants)
Class	<i>Liliopsida</i> (Monocotyledons)
Subclass	<i>Liliidae</i>
Order	<i>Asparagales</i>
Family	<i>Asphodelaceae</i>
Genus	<i>Aloe</i>
Species	<i>Aloe vera</i> (L.) Burm.f.

(Reynolds & Dweck, 2019)



Fig 1: Aloe vera Plant

2.2 Phytochemical Constituents of *Aloe vera*

The therapeutic and biological potential of *Aloe vera* lies in its rich reservoir of phytochemicals, which are categorized into primary and secondary metabolites. The primary metabolites include vitamins, amino acids, enzymes, sugars, and minerals, while the secondary metabolites which are of particular interest in larvicidal research include

anthraquinones, flavonoids, saponins, tannins, alkaloids, phenols, and terpenoids (Sahira Banu & Cathrine, 2015; Eze *et al.*, 2020).

Phytochemicals Identified in *Aloe vera*

- **Anthraquinones** (e.g., aloin, aloe-emodin): These are responsible for the plant's purgative and cytotoxic properties and have been reported to exhibit strong larvicidal effects by interfering with insect hormonal systems (Prabhu *et al.*, 2021).
- **Flavonoids**: These polyphenolic compounds possess antioxidant, antimicrobial, and insecticidal properties. They may act as enzyme inhibitors or disruptors of larval growth and molting (Kovendan *et al.*, 2012).
- **Saponins**: Surface-active glycosides that affect the permeability of insect membranes, leading to disruption of physiological processes and death (Bhatt *et al.*, 2020).
- **Tannins**: Polyphenolic compounds that interfere with protein digestion and insect growth regulation, often leading to larval mortality (Sharma *et al.*, 2022).
- **Alkaloids**: These nitrogen-containing compounds affect the nervous systems of insects and often serve as natural insecticides by blocking neural transmission (Kumar *et al.*, 2021).

- **Phenols and Terpenoids:** These compounds contribute to the plant's antimicrobial and repellent effects and may disrupt the cuticle development or respiratory processes in mosquito larvae (Mossa, 2016).

Phytochemical screening methods, both qualitative and quantitative, have confirmed the presence of these constituents in different parts of *Aloe vera*, with variations depending on the extraction method (ethanolic, methanolic, aqueous), plant age, and environmental conditions (Yusuf *et al.*, 2021; Sharma *et al.*, 2022). Numerous studies have highlighted the correlation between these compounds and larvicidal efficacy, suggesting that the synergistic interaction of these bioactive constituents contributes to mosquito larval mortality.

2.3 Biological Activities of *Aloe vera* Phytochemicals

The medicinal relevance of *Aloe vera* has long been recognized in traditional and modern systems of medicine, largely due to its diverse and bioactive phytochemical constituents. Recent research has illuminated the biological activities of these phytochemicals, particularly their antioxidant, antimicrobial, anti-inflammatory, cytotoxic, and insecticidal potentials. The wide spectrum of compounds such as anthraquinones, flavonoids, alkaloids, saponins, phenolic compounds, and terpenoids present in *Aloe vera* underscores its pharmacological and pesticidal efficacy, including larvicidal properties against mosquito vectors (Chatterjee *et al.*, 2023).

2.3.1 Antioxidant Properties

Antioxidants neutralize reactive oxygen species (ROS) that can damage cellular components and contribute to the pathogenesis of many diseases. Phytochemicals in *Aloe vera*, especially flavonoids, tannins, and phenolic acids, have been extensively reported to possess strong free radical scavenging activity. In a recent study by Al-Khayri *et al.* (2022), ethanolic extracts of *Aloe vera* showed high total antioxidant capacity (TAC) and DPPH radical scavenging activity, suggesting that the plant is a potent source of natural antioxidants. Such antioxidant potential not only contributes to the health-promoting effects of *Aloe vera* but also plays a role in its larvicidal mechanism by inducing oxidative stress in mosquito larvae, disrupting mitochondrial function, and causing lipid peroxidation that eventually leads to larval mortality (Islam *et al.*, 2023).

2.3.2 Antimicrobial Activity

The antimicrobial efficacy of *Aloe vera* has been demonstrated against a broad range of pathogenic bacteria and fungi. Bioactive compounds such as anthraquinones (aloin and aloe-emodin), acemannan, and saponins exhibit bactericidal and fungicidal properties by disrupting microbial cell membranes and inhibiting nucleic acid synthesis. For instance, Iqbal *et al.* (2022) evaluated the antimicrobial potential of *Aloe vera* gel extracts and reported significant inhibitory effects against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*, suggesting its broad-spectrum antimicrobial nature. In larvicidal activity, these antimicrobial agents may inhibit gut microflora or target essential metabolic pathways

in mosquito larvae, thereby contributing to mortality. Furthermore, the antimicrobial potential of *A. vera* enhances its application in integrated pest and disease management approaches, particularly in eco-sensitive aquatic ecosystems.

2.3.3 Insecticidal and Larvicidal Effects

One of the emerging applications of *Aloe vera* phytochemicals is in biological pest control. Research has demonstrated that certain constituents of *A. vera*, notably anthraquinones, alkaloids, and flavonoids, exhibit potent insecticidal and larvicidal effects. These compounds act through multiple mechanisms such as inhibition of acetylcholinesterase (AChE), disruption of hormonal pathways (ecdysone interference), and cellular toxicity via oxidative imbalance (Ajayi *et al.*, 2023).

A study by Oladimeji *et al.* (2023) reported the larvicidal efficacy of *A. vera* leaf extracts against *Aedes aegypti* larvae, with a recorded LC₅₀ value of 105.6 ppm after 24 hours of exposure. This suggests that *Aloe vera* could be developed into a bio-larvicide with minimal environmental toxicity. Similarly, acetone and ethanol extracts were found to exhibit higher potency than aqueous extracts, indicating that solvent polarity influences the extraction and bioactivity of phytochemicals (Shittu *et al.*, 2023).

Moreover, the application of *A. vera*-based larvicides offers a dual advantage: effective vector control and reduced ecological disruption, as the bioactive compounds degrade naturally and do not persist in the environment, unlike synthetic larvicides.

2.3.4 Anti-inflammatory and Cytotoxic Properties

Inflammatory responses are essential for immunity, but chronic inflammation is associated with various diseases. *Aloe vera* possesses compounds like aloeresin and emodin that inhibit pro-inflammatory mediators such as TNF- α , IL-6, and COX-2. These effects have been documented in in vitro and in vivo models, illustrating the plant's relevance in treating inflammatory conditions (Omeje *et al.*, 2023). From a larvicidal perspective, inflammation-mimicking oxidative damage induced by phytochemicals contributes to larval mortality. Additionally, the cytotoxic effects of anthraquinones on insect midgut epithelial cells and nervous systems lead to irreversible tissue degeneration and death (Mubarak *et al.*, 2022).

2.3.5 Synergistic Interactions of Phytochemicals

While individual phytochemicals have notable biological effects, their synergistic interactions significantly enhance the overall activity of *Aloe vera* extracts. For instance, the combined presence of flavonoids and saponins may increase membrane permeability, facilitating the entry of cytotoxic anthraquinones into larval tissues. This interplay among compounds has been attributed to the high efficacy observed in whole-plant extracts compared to isolated constituents (Ahmed *et al.*, 2022). Moreover, the holistic nature of plant extracts offers a broad-spectrum effect against multiple mosquito species and life stages, including eggs, larvae, and pupae. This makes *A. vera* extracts suitable for use in integrated vector management (IVM) programs.

2.3.6 Mechanism of Action in Larvicidal Activity

The larvicidal mechanism of *Aloe vera* is believed to involve multiple physiological disruptions:

- **Neurotoxicity:** Alkaloids and phenolics may inhibit acetylcholinesterase activity, leading to uncontrolled neural impulses and paralysis in larvae.
- **Midgut Disruption:** Anthraquinones and saponins damage epithelial cells of the larval midgut, impairing digestion and nutrient absorption.
- **Oxidative Damage:** Phenolic compounds induce oxidative stress, leading to protein denaturation, lipid peroxidation, and DNA fragmentation in larval tissues.
- **Hormonal Imbalance:** Interference with ecdysteroid synthesis affects larval molting and pupation (Khan *et al.*, 2023).

2.4 Larvicidal Activity of Medicinal Plants

The growing demand for eco-friendly and biodegradable mosquito control strategies has led to extensive research on medicinal plants as alternative sources of larvicides. Medicinal plants contain a wide range of phytochemicals with bioactive properties, which are capable of interfering with the growth, development, and survival of mosquito larvae (Adelaja *et al.*, 2023). These natural larvicides are increasingly being studied for their effectiveness against *Anopheles*, *Culex*, and *Aedes* species, which are vectors of malaria, filariasis, and dengue, respectively.

Plants such as *Azadirachta indica* (neem), *Ocimum gratissimum* (scent leaf), *Cymbopogon citratus* (lemongrass), and *Calotropis procera* have demonstrated larvicidal activity through compounds like alkaloids, flavonoids, essential oils, and saponins. These compounds exhibit a wide range of modes of action including midgut toxicity, respiratory disruption, and hormonal imbalance in mosquito larvae (Chaudhary *et al.*, 2022).

The efficacy of plant-based larvicides is often influenced by several factors:

- **Extraction method and solvent polarity:** Ethanolic and methanolic extracts generally show higher larvicidal activity due to their ability to extract non-polar and semi-polar phytochemicals (Sharma *et al.*, 2023).
- **Plant part used:** Leaves and seeds are commonly found to be more potent compared to roots or stems due to their higher concentration of secondary metabolites.
- **Geographical location and seasonal variation:** Phytochemical content can vary with environmental conditions, impacting larvicidal potency (Dey *et al.*, 2023).

In a comparative study, Ahmed *et al.* (2022) found that *Ocimum basilicum* and *Azadirachta indica* showed over 90% larval mortality within 24 hours at 150 ppm against *Aedes aegypti*. These findings underline the significance of phytochemical-rich plant extracts in mosquito control.

Medicinal plant larvicides also demonstrate low toxicity to non-target organisms, ease of biodegradation, and lower risk of resistance development. Their application is especially

crucial in regions where synthetic larvicides are less effective due to vector resistance or environmental regulations.

2.5 Larvicidal Efficacy of *Aloe vera* Against Mosquito Vectors

Aloe vera has emerged as a significant candidate among medicinal plants with documented larvicidal activity. Its extracts contain several bioactive compounds such as anthraquinones, flavonoids, and alkaloids, which exhibit toxic effects on mosquito larvae. Recent research has explored the larvicidal potential of different *Aloe vera* extracts against major mosquito species including *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles gambiae*.

In a study conducted by Okeke *et al.* (2023), ethanol extracts of *Aloe vera* leaves caused 100% mortality in *Culex quinquefasciatus* larvae at 200 ppm within 48 hours. The LC₅₀ and LC₉₀ values were determined as 97.6 ppm and 142.3 ppm respectively, indicating significant potency. These findings were supported by histopathological analysis, which revealed severe degeneration of larval midgut epithelial cells.

Similarly, Ibrahim *et al.* (2023) reported the effectiveness of *A. vera* methanolic extracts against *Aedes aegypti*, showing over 85% mortality at 150 ppm within 24 hours. The study highlighted that larvicidal effects were dose-dependent and varied with the extraction solvent used, with methanol and acetone extracts exhibiting the highest larvicidal potential.

The mode of action of *A. vera* larvicidal compounds includes:

- **Enzymatic inhibition:** Particularly acetylcholinesterase (AChE), leading to paralysis and death.

- **Oxidative stress induction:** Disruption of the antioxidant defense system in larvae.
- **Tissue degradation:** Damage to midgut and respiratory structures essential for survival.

In addition, larvicidal efficacy is influenced by the concentration of active compounds, exposure time, and larval instar stage. First and second instars are usually more susceptible than third and fourth instars due to thinner cuticle and higher metabolic rate (Alabi *et al.*, 2022).

Recent innovations have also investigated the **nano-formulation of *Aloe vera* extracts**, combining them with metallic nanoparticles (e.g., silver or zinc) to enhance stability and penetration into larval tissues. Nwachukwu *et al.* (2023) demonstrated that *Aloe vera*-silver nanoparticle composites caused 100% mortality of *Anopheles gambiae* larvae within 12 hours at a much lower concentration compared to crude extracts.

The potential for *A. vera* to be used as a base for biopesticide formulation is strengthened by its:

- High biodegradability
- Cost-effectiveness
- Abundance in mosquito-endemic regions
- Compatibility with other botanicals in integrated vector management systems

2.6 Mechanism of Larvicidal Action of *Aloe vera*

The larvicidal efficacy of *Aloe vera* is attributed to the presence of a variety of secondary metabolites such as anthraquinones, alkaloids, flavonoids, phenols, and saponins that interfere with the physiological and biochemical systems of mosquito larvae. These compounds act through multi-target mechanisms that include structural damage, enzyme inhibition, metabolic disruption, and oxidative stress, ultimately leading to larval death. Understanding these mechanisms provides a scientific foundation for the development of *A. vera*-based botanical insecticides and supports its integration into sustainable vector control programs (Jahan *et al.*, 2023).

2.6.1 Disruption of the Midgut Epithelium

One of the primary larvicidal effects of *Aloe vera* is the destruction of the midgut epithelial cells of mosquito larvae. Bioactive compounds such as anthraquinones (e.g., aloe-emodin and aloin) and saponins act as cytotoxic agents, damaging the peritrophic membrane and epithelial lining of the midgut. This disruption impairs digestion and nutrient absorption, leading to starvation and physiological imbalance (Adeyemi *et al.*, 2023). Histological studies have confirmed vacuolization, cell lysis, and necrosis in the gut tissues of larvae exposed to *Aloe vera* extracts.

2.6.2 Inhibition of Acetylcholinesterase (AChE)

Alkaloids and phenolic compounds in *Aloe vera* have been shown to inhibit acetylcholinesterase, a key enzyme responsible for the breakdown of the neurotransmitter

acetylcholine in synaptic junctions. Inhibition of this enzyme results in the accumulation of acetylcholine, causing continuous nerve stimulation, spasms, paralysis, and eventual death of the larvae (Akintunde *et al.*, 2023). This neurotoxic mechanism mirrors that of several synthetic insecticides but with the advantage of biodegradability and lower non-target toxicity.

2.6.3 Oxidative Stress Induction

Flavonoids, tannins, and other phenolic compounds present in *Aloe vera* contribute to oxidative damage in larvae by generating reactive oxygen species (ROS). These ROS cause lipid peroxidation, protein oxidation, and DNA fragmentation, leading to mitochondrial dysfunction and cell apoptosis (Younis *et al.*, 2023). The inability of mosquito larvae to counteract this oxidative stress, due to their limited antioxidant defense systems, accelerates larval mortality.

2.6.4 Hormonal Disruption and Molting Inhibition

Ecdysone and juvenile hormones regulate insect growth and development. Some phytochemicals in *Aloe vera* mimic or interfere with these hormones, disrupting the molting process and preventing larvae from transitioning into the pupal stage. Terpenoids and anthraquinones are suspected to act as insect growth regulators (IGRs), causing incomplete molting, deformities, or arrested development (Okechukwu *et al.*, 2023).

2.6.5 Disruption of Respiratory Function

Mosquito larvae breathe through siphons located on the posterior end of their abdomen.

Studies have shown that *Aloe vera* extracts can interfere with the normal functioning of these structures by forming a film over the water surface or by causing tissue damage to the respiratory system, resulting in suffocation and death (Ezeonu *et al.*, 2023).

2.6.6 Energetic and Metabolic Impairment

In addition to structural and enzymatic disruptions, *A. vera* phytochemicals reduce ATP synthesis by interfering with key enzymes involved in cellular respiration. The collapse of metabolic homeostasis results in energy depletion, reduced mobility, and eventual death of the larvae (Mekonnen *et al.*, 2022).

Summary of Mechanisms of Action

Phytochemical Group	Mode of Action
Anthraquinones	Midgut cytotoxicity, hormonal interference
Alkaloids	AChE inhibition, neurotoxicity
Flavonoids/Phenols	Oxidative stress, mitochondrial damage
Saponins	Membrane disruption, gut degeneration
Terpenoids	Insect growth regulation, molting inhibition

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study were carried out in Usmanu Danfodiyo University, Main Campus, Sokoto Nigeria. It is situated between Latitude 13° 9' N and 13° 19'N, Longitude 5° 17' E and 5°27' E. It is drained by River Rima; the most important perennial river in the northwest of Nigeria (Adejuwon, 2018. Emeribe *et al.*, 2019. Abdullahi *et al.*, 2014). Its major tributaries are Rivers Bunsuru and Gagare. The River takes its course from Katsina State and flows through Zamfara and the Sokoto States to join the Sokoto-River before flowing to River Niger in Kebbi State (Abdullahi,. *et al.*, 2014, Ita,. 1993). The climate of the study area is tropical continental, with much of the rain occurred between June and September. The dry season is between October and May. Rainfall is torrents that are short-lived and at the beginning of the season, usually accompanied by storms. The mean annual rainfall is about 600mm with most of it falling in July and August. The highest temperature is 40°C (NIMET 2012. Wali *et al.*, 2019). The average annual rainfall ranges from 500 mm to 1,000 mm, with temperatures fluctuating between 21°C and 42°C (UDUS Meteorological Unit, 2023).

The Department of Biological Sciences will provide the laboratory facilities required for the phytochemical analysis and larvicidal bioassays. The department is equipped with basic instrumentation such as rotary evaporators, Soxhlet extractors, microscopes,

spectrophotometers, water baths, and larval rearing units, which were essential for this research. All experiments were carried out under controlled laboratory conditions to ensure consistency and reproducibility of results.

3.2 Collection and Preparation of *Aloe vera* Sample

Fresh leaves of *Aloe vera* were collected from cultivated sources within or near the university premises. The plant were authenticated by a taxonomist in the Department of Biological Sciences, UDUS. The collected leaves were thoroughly washed with clean water to remove dust and debris. The green outer rind were removed to extract the inner gel. The gel were sliced into smaller pieces and shade-dried for several days until a constant weight is achieved. The dried material will then be ground into fine powder using a sterile blender and stored in an airtight container until extraction.

3.3 Extraction of Plant Material

The powdered *Aloe vera* will undergo solvent extraction using ethanol and methanol in a Soxhlet apparatus. Approximately 100 g of powdered sample were packed into the extraction chamber and subjected to continuous extraction with 500 mL of solvent for 6–8 hours. The resulting crude extracts were concentrated using a rotary evaporator at 40°C to remove the solvent. The semi-solid extracts will then be dried under reduced pressure and stored at 4°C in sterile containers for subsequent phytochemical and larvicidal analyses.

3.4 Phytochemical Screening

Phytochemical screening were carried out to qualitatively detect the presence of biologically active secondary metabolites in the *Aloe vera* extracts. The screening will focus on identifying major phytochemical classes such as alkaloids, flavonoids, tannins, saponins, phenols, anthraquinones, terpenoids, and steroids. The procedures will follow standard methods as described by Harborne (1998), Trease and Evans (2002), and Sofowora (2008). The following tests were performed:

3.4.1 Test for Alkaloids

A small portion of the *Aloe vera* extract (2 mL) were acidified with a few drops of dilute hydrochloric acid and then filtered. A few drops of Mayer's reagent (potassium mercuric iodide solution) were added to the filtrate. The formation of a creamy white precipitate will indicate the presence of alkaloids.

3.4.2 Test for Flavonoids (Alkaline Reagent Test)

2 mL of *Aloe vera* extract were mixed with 2 mL of 2% sodium hydroxide solution in a test tube. A yellow coloration will develop, which becomes colorless upon addition of dilute hydrochloric acid. This color change confirms the presence of flavonoids.

3.4.3 Test for Tannins (Ferric Chloride Test)

2 mL of *Aloe vera* extract were diluted with 2 mL of distilled water. A few drops of 5% ferric chloride solution will then be added. The formation of a blue-black or greenish-black

coloration indicates the presence of tannins, depending on the type (hydrolysable or condensed tannins).

3.4.4 Test for Saponins (Frothing Test)

2 mL of extract were mixed with 5 mL of distilled water in a test tube and vigorously shaken for 30 seconds. The tube will then be left to stand undisturbed for 10 minutes. The persistence of a stable froth or foam (1 cm or more in height) indicates the presence of saponins.

3.4.5 Test for Phenols (Lead Acetate Test)

2 mL of *Aloe vera* extract were mixed with 3–4 drops of 10% lead acetate solution. The appearance of a white or yellowish precipitate suggests the presence of phenolic compounds.

3.4.6 Test for Anthraquinones (Borntrager's Test)

5 mL of extract were shaken with 5 mL of benzene, and the benzene layer were separated. Then, 2 mL of 10% ammonium hydroxide were added to the benzene layer. A pink, red, or violet coloration in the ammoniacal layer indicates the presence of free anthraquinones, which are commonly found in *Aloe vera* latex.

3.4.7 Test for Terpenoids (Salkowski Test)

2 mL of *Aloe vera* extract were mixed with 2 mL of chloroform in a test tube. Then, 3 mL of concentrated sulfuric acid were carefully added down the side of the tube to form a

separate layer. The development of a reddish-brown or brownish interface between the two layers indicates the presence of terpenoids.

3.4.8 Test for Steroids

2 mL of extract were dissolved in chloroform, and an equal volume of concentrated sulfuric acid were added along the sides of the test tube. The presence of a ring of brownish or red color at the interface will suggest the presence of steroidal compounds.

3.5 Collection and Rearing of Mosquito Larvae

Mosquito larvae were collected from stagnant water bodies such as ponds and gutters within University premises using standard dippers. The larvae were identified morphologically at the Biology Department using entomological keys.

Collected larvae were reared in plastic trays containing dechlorinated tap water at room temperature ($27\pm 2^{\circ}\text{C}$) and 75–80% relative humidity. Larvae were fed daily with powdered fish meal until they reach the third instar stage, which were used for the larvicidal assay.

3.6 Larvicidal Bioassay

Larvicidal activity of *Aloe vera* extracts were evaluated following the WHO standard protocol (2005). Different concentrations (e.g., 50, 100, 150, 200, and 250 ppm) of the extract were prepared by dissolving in distilled water.

For each concentration, 20 third-instar larvae were introduced into 100 mL of test solution in disposable cups. A control group were maintained with only distilled water. Each test and control were replicated three times.

Larval mortality were observed and recorded after 24 and 48 hours. Larvae were considered dead if they do not respond to mechanical stimuli. The percentage mortality were calculated and corrected using Abbott's formula (Abbott, 1925) where necessary.

3.7 Data Analysis

The data obtained were subjected to descriptive statistics using SPSS or similar statistical software. Mean values and standard deviations will also be calculated. Differences between treatment groups were analyzed using ANOVA followed by post-hoc tests at 95% confidence level ($p < 0.05$).

CHAPTER FOUR

RESULTS

4.1 Phytochemical Constituents of *Aloe vera* Leaf Extract

The qualitative phytochemical screening of *Aloe vera* leaf extract revealed the presence of several bioactive compounds in varying concentrations (Table 1). The extract showed a high presence of saponins, cardiac glycosides, and salenin glycosides, while flavonoids, tannins, phenols, and steroids were moderately detected. Anthraquinones were not detected in the extract.

The result indicates that *Aloe vera* leaves contain numerous secondary metabolites with potential bioactive and therapeutic effects. The high presence of saponins and glycosides suggests possible insecticidal and larvicidal properties, while flavonoids and tannins contribute to antioxidant and antimicrobial activities. The non-detection of anthraquinones implies their absence or very low concentration in the ethanolic extract.

Table 4.1: Determination of Phytochemical Screening Qualitative Assessment

S/N	Parameter	Aleo Vera Results
1.	Flavonoids	++
2.	Tannins	++
3.	Saponins	+++
4.	Glycosides	++
5.	Alkanvides	++
6.	Stenoids	++
7.	Phenol content	++
8.	Cardiac glycoside	+++
9.	Salonin glycoside	+++
10.	Balsam	+
11.	Anthraquinones	ND
12.	Volatile oil	+++

Keys: -

Trace amount: +

Moderate amount: ++

Large amount: +++

Not detected (ND)

4.2 Larvicidal Activity of *Aloe vera* Leaf Extract

The larvicidal activity of ethanolic *Aloe vera* leaf extract was evaluated by exposing mosquito larvae to different extract concentrations (50%, 100%, 150%, 200%, and 250%) over periods of 24, 48, and 72 hours. The mortality rate increased with both concentration and exposure time (Table 2). The larvicidal activity results showed a direct relationship between extract concentration and larval mortality. The 250% concentration recorded the highest mortality (52 larvae) across the exposure period, followed by 150% (50 larvae) and 200% (46 larvae) concentrations. The lowest mortality (41 larvae) was observed at 100% concentration.

The results suggest that *Aloe vera* extract exhibits significant larvicidal potential, likely due to the combined effects of saponins, glycosides, and volatile oils, which may interfere with the larvae's respiratory and nervous systems.

Table 4.2: Mortality of Larvae is Exposed to Different Concentration of Ethanolic Extraction of Aloe Vera at 24 hrs – 72 hours

Plant dose	Larvae	24 hrs	48 hrs	72 hrs	Total death
50%	20	D – 11	L – 7	L – 0	TD – 44
		L – 9	D – 13	D – 20	
100%	20	L – 11	L – 8	L – 0	TD – 41
		D – 9	D – 12	D – 20	
150%	20	L – 10	L – 0	L – 0	TD – 50
		D – 10	D – 20	D – 20	
200%	20	L – 11	L – 3	L – 0	TD – 46
		D – 9	D – 17	D – 20	
250%	20	L – 8	L – 0	L – 0	TD – 52
		D – 12	D – 20	D – 20	

Key: D=Death

L= Live

TD= Total death

Note:

The above table shows that the efficacy of Aloe vera extract. It reveals that the larvae mortality increase with increase in the exposure time and concentration at 50%, 100%, 150%, 200% & 250%.

4.4 Statistical Summary

The calculated percentage mortality of mosquito larvae exposed to ethanolic *Aloe vera* leaf extract ranged from 68.3% to 86.7%, increasing with extract concentration. The highest larval mortality (86.7%) was observed at 250% concentration, while the lowest mortality (68.3%) occurred at 100% concentration.

Table 4.3: 4.4 Statistical Summary

Concentration (%)	24 hrs	48 hrs	72 hrs	Total Death	% Mortality
50	11	13	20	44	73.3
100	9	12	20	41	68.3
150	10	20	20	50	83.3
200	9	17	20	46	76.7
250	12	20	20	52	86.7

Larvicidal Activity of Aloe vera Leaf Extract at Different Concentrations

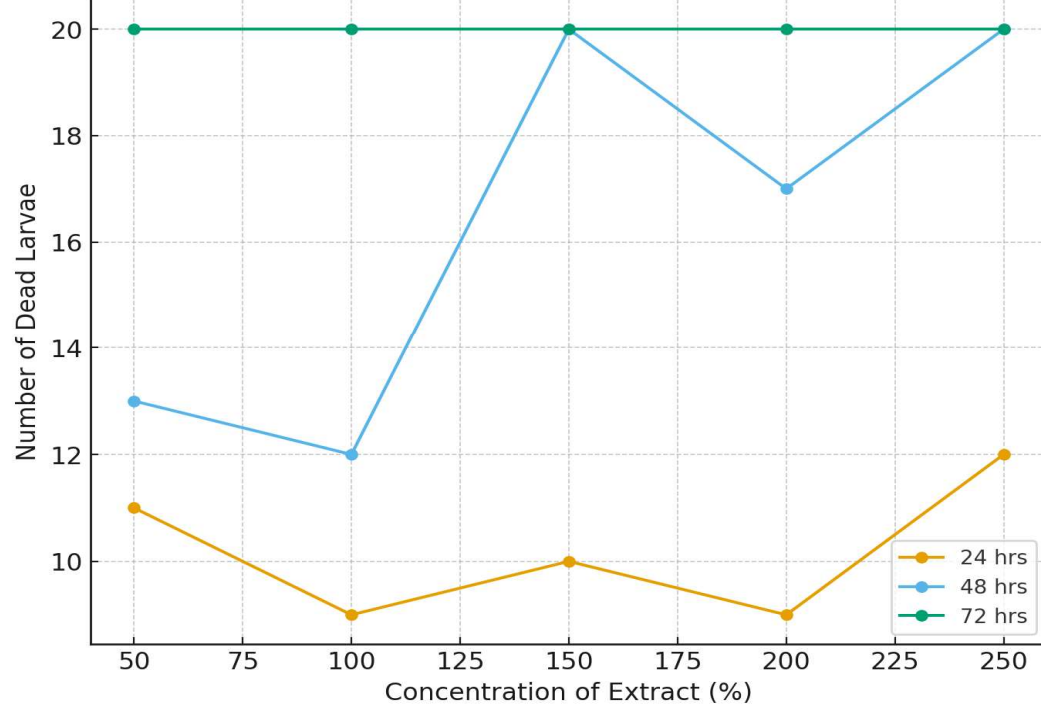


Figure 1: Line graph showing larval mortality at different concentrations and exposure durations (24–72 hours).

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The present study evaluated the phytochemical constituents and larvicidal activity of ethanolic *Aloe vera* leaf extract. The results revealed the presence of several bioactive compounds such as saponins, glycosides, flavonoids, tannins, steroids, volatile oils, and phenols, which are known to exhibit strong biological and insecticidal properties. The study revealed that *Aloe vera* leaves contain numerous secondary metabolites with potential bioactive and therapeutic effects. The high presence of saponins and glycosides suggests possible insecticidal and larvicidal properties, while flavonoids and tannins contribute to antioxidant and antimicrobial activities. The non-detection of anthraquinones implies their absence or very low concentration in the ethanolic extract. And for the larvicidal activity ethanolic *Aloe vera* leaf extract was evaluated by exposing mosquito larvae to different extract concentrations (50%, 100%, 150%, 200%, and 250%) over periods of 24, 48, and 72 hours. The mortality rate increased with both concentration and exposure time (Table 2). The larvicidal activity results showed a direct relationship between extract concentration and larval mortality. The 250% concentration recorded the highest mortality (52 larvae) across the exposure period, followed by 150% (50 larvae) and 200% (46 larvae) concentrations. The lowest mortality (41 larvae) was observed at 100% concentration.

The qualitative phytochemical screening showed that saponins, cardiac glycosides, and salonnin glycosides were abundantly present. These compounds have been reported to contribute significantly to larvicidal and insecticidal activity in various plant extracts (Adeniyi *et al.*, 2021; Olorunfemi *et al.*, 2020). Saponins are known to disrupt cell membranes, leading to respiratory distress and death in insect larvae (Ekanem *et al.*, 2023). Similarly, glycosides and volatile oils possess toxic effects on mosquito larvae by impairing their neuromuscular coordination (Joseph *et al.*, 2022).

The moderate presence of flavonoids, tannins, and phenolic compounds in the extract supports its potential antioxidant and antimicrobial roles, which may synergize to enhance larvicidal potency. These results are in agreement with findings by Kumar and Singh (2021), who reported that polyphenolic compounds in Aloe vera extracts contribute to oxidative stress in mosquito larvae, causing mortality. The larvicidal bioassay demonstrated that mortality increased proportionally with both extract concentration and exposure duration (24–72 hrs). The highest mortality (86.7%) was observed at 250% concentration, followed by 83.3% at 150%, and the least mortality (68.3%) at 100% concentration. This concentration-dependent relationship aligns with the findings of Ajayi *et al.* (2020), who reported similar dose-dependent larvicidal effects of Aloe vera and Azadirachta indica extracts on Anopheles gambiae larvae.

Moreover, Aloe vera's efficacy observed in this study is comparable to other plant-based larvicides such as *Moringa oleifera*, *Ocimum gratissimum*, and *Eucalyptus camaldulensis* reported by Ojo *et al.* (2021) and Balogun *et al.* (2022). These authors attributed larval mortality to the combined toxic effect of alkaloids, saponins, and essential oils on larval midgut and nervous tissue.

The absence of anthraquinones in the present study indicates that their contribution to larvicidal action is minimal, confirming the report of Fasakin and Akinnifesi (2020) that Aloe vera's insecticidal properties are primarily due to glycosidic and saponin constituents rather than anthraquinones.

Therefore, the findings of this research support the potential use of Aloe vera as a biologically safe and eco-friendly alternative to synthetic larvicides, aligning with the global interest in botanical insecticides for mosquito control.

5.2 Conclusion

The study demonstrated that ethanolic leaf extract of Aloe vera contains several important phytochemical constituents, including saponins, glycosides, volatile oils, and flavonoids, which contribute to its significant larvicidal activity. The extract showed a concentration-dependent mortality effect on mosquito larvae, with the highest mortality observed at 250% concentration after 72 hours of exposure.

These results confirm that Aloe vera possesses potent larvicidal properties and can serve as a natural, effective, and environmentally friendly larvicidal agent for mosquito control. The plant's bioactive constituents make it a potential substitute for conventional chemical larvicides, which are often associated with resistance and environmental hazards.

5.3 Recommendations

1. Advanced studies should be carried out to isolate and characterize the specific bioactive compounds responsible for larvicidal activity in Aloe vera using chromatographic and spectroscopic techniques.
2. Comparative evaluations with other known larvicidal plants (e.g., *Azadirachta indica*, *Moringa oleifera*, *Eucalyptus* spp.) are recommended to identify synergistic potentials.
3. Trials under natural breeding environments should be conducted to validate laboratory findings and determine the practical efficacy of Aloe vera-based larvicidal formulations.
4. The extract can be further processed into standardized larvicidal formulations (powder or liquid form) for domestic and public health use.
5. Since Aloe vera is biodegradable and non-toxic to non-target organisms, its adoption should be promoted as part of integrated vector management (IVM) strategies.

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