

**EVALUATION OF THE ACARICIDAL ACTIVITY OF THE CRUDE VOLATILE OIL AND  
FRACTIONS OF *Ocimum gratissimum* LEAF AGAINST TICKS**



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**BENIN CITY**

**EDO STATE**

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF PHARMACOGNOSY,  
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**CERTIFICATION**

This is to certify that this project work was carried out by OLAWUNMI HABEEBAT OMOBOLANLE (PHA1908571) in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City.

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## **DEDICATION**

This work is dedicated to God Almighty, for His infinite mercies and guidance throughout my journey in School of Pharmacy.

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## ABSTRACT

In tropical and subtropical areas, tick infestations are a serious hazard to public health and livestock output. These ectoparasites act as vectors for a variety of zoonotic illnesses in addition to causing anemia, skin irritation, and secondary infections in animals. Due of extensive multi-drug resistance, environmental contamination, and public health issues such residues in meat and milk, conventional chemical acaricides are failing. Because of its antibacterial, insecticidal, and repellent properties, *Ocimum gratissimum*, often referred to as smell leaf, is widely used in West African ethnomedicine. Using a ~~elevenger~~Clevenger apparatus, the volatile oil was extracted from fresh *Ocimum gratissimum* leaves. Column chromatography and TLC were then used to fractionate and isolate the oil. The live cow ticks were treated with 20  $\mu$ L of various oil concentrations (1%, 2%, 5%, and 10%). To assess effectiveness, tick mortality was tracked over a 24-hour period. The volatile oil's effectiveness as a deadly agent against the tick genus *Hyalomma rufipes* was confirmed when it reached 100% mortality at 10% concentration. The oil maintained a high mean mortality of  $86.67 \pm 15.28$  even at the lowest concentration tested (1%). At concentrations of 2%, 5%, and 10%, the oil completely killed *Rhipicephalus microplus* in every replicate. A significant mean mortality of  $93.3 \pm 11.55$  was obtained even at the lowest concentration tested (1%). *Ocimum gratissimum* volatile oil is a particularly good option for developing an integrated tick management product that is eco-friendly, efficient, and sustainable.

## INTRODUCTION

Ticks (Acari: Ixodidae) represent an essential category of ectoparasites, inflicting considerable morbidity and mortality in both livestock and humans globally. In this sense, they are surpassed only by mosquitoes (Dossou 2023). Babesiosis, theileriosis, and ehrlichiosis are only a few of the serious zoonotic and veterinary illnesses that ticks are known to transmit. Reduced production and high control costs have an annual economic impact on agriculture of billions of dollars, especially in tropical and subtropical regions where species like *Rhipicephalus microplus* thrive (Wang 2025).

Synthetic chemical acaricides, such as organophosphates and synthetic pyrethroids, have been widely used to control ticks for decades. Widespread tick resistance is a major and expanding problem brought on by the abuse and overuse of these pesticides (Benelli and Pavela, 2021). Significant environmental issues are also brought up by these artificial compounds. Meat, milk, and the environment all contain residues. Researchers and regulatory bodies are now looking for safer and more environmentally friendly options as a result (Wang 2025). Interest in botanical acaricides has increased due to the urgent demand for efficient, biodegradable, and environmentally friendly control agents.

Essential oils (Essential oils) are concentrated, hydrophobic liquids that are sometimes referred to as volatile oils or plant essences. They include volatile aromatic chemicals derived from various plant sources (Hegazy 2025). Essential oils are a promising class of natural chemicals because they are complex combinations of volatile secondary metabolites generated from aromatic plants (Isman, 2020).

From a pharmacognosy perspective, Essential oils offer two primary benefits: rapid biodegradability and a variety of frequently synergistic activities against target pests that complicate the development of resistance (Benelli and Pavela, 2021). They provide a variety of functions, such as communication and defense. The primary ecological function of volatile oils is to protect plants from microorganisms and herbivores by acting as a chemical immune system (Nayak 2025). They possess

both antifungal and antibacterial properties. Essential oils are housed in unique structures, such as *Ocimum's* glandular trichomes, and are released when the plant sustains harm. Scent leaf contains phenolic components such eugenol and thymol, as well as a variety of monoterpenes, which are powerful agents that interfere with bacterial and fungal cell membranes and enzyme functioning (Kowalczyk 2024; Nayak 2025).

Essential oils have the ability to function as direct contact poisons against mites and insects. They can swiftly penetrate the outer layer of arthropods and interfere with their nervous system, digestive tract, or endocrine systems since their volatile components are highly lipophilic, which frequently leads to immediate immobilization or death (Senthil-Nathan 2020).

Essential oils play a crucial role in the chemical exchange between plants and their environment. These aerial signals are essential for both reproduction and survival. For example, flower essential oils draw pollinators that are necessary for reproduction, such as moths and bees (Nayak 2025). Essential oils can deter pests as well. Generalist herbivores are frequently discouraged from important plant portions by strong scents. One significant benefit of using botanical insecticides is their repelling effect. As an indirect defense, Essential oils can also draw in natural enemies. When a herbivore damages a plant, it frequently releases a distinct collection of Herbivore-Induced Plant Volatiles (HIPVs) that alert the herbivore's natural enemies, including parasitic wasps or predatory mites. In order to reduce the effects of herbivores, this effectively recruits "bodyguards" (Senthil-Nathan 2020). Essential oils cause damaged plants to generate volatile signals that other plants of the same or different species can detect. They are prompted to "prime" their own defenses as a result. When attacked later, they react more swiftly and forcefully as a result (Kowalczyk 2024). Plants may experience oxidative stress during periods of high temperatures or drought.

By stabilizing cell membranes and neutralizing dangerous Reactive Oxygen Species (ROS), volatile isoprenoids like isoprene and monoterpenes contribute to the photosynthetic systems' increased temperature tolerance (Hara, 2020). Plants, particularly those in the Lamiaceae family, frequently

increase their levels of secondary metabolites, such as Essential oils, when they are under mild drought stress. This may be a survival strategy to increase stress tolerance and reduce water loss (Hosseini R, 2025).

## **1.1 Methods Of Extracting Essential Oils**

Essential oils are extracted using a variety of methods, each with varying degrees of effectiveness, scalability, and selectivity.

1. The most used industrial technique is steam distillation, often known as direct steam distillation. The aromas are vaporized by pressurized steam, condensed, and separated in this procedure (Sadgrove and Jones 2015).
2. The process of hydro distillation entails submerging plant material in boiling water. The oil is transported by the steam and then condenses. Heat-sensitive components may be harmed by this straightforward procedure (Srivastava and Mathur 2022).
3. Organic solvents like hexane or enfleurage are used in solvent extraction. Although it works well for subtle flower fragrances, its high cost and solvent residues prevent widespread application (Srivastava and Mathur 2022).
4. Microwave-assisted distillation, pressured liquid extraction, and carbon dioxide (CO<sub>2</sub>) extraction, commonly referred to as supercritical fluid extraction, are examples of contemporary techniques that increase yield and safeguard delicate substances. But they frequently only function in analytical or pilot-plant environments (Srivastava and Mathur 2022).

### **1.1.1 Working Principle Of Clevenger's Apparatus In Steam Distillation**

One particular kind of glassware used for continuous hydrodistillation is the Clevenger-type equipment. Based on density, it extracts volatile oil and separates it from the watery distillate (hydrosol). A continuous cycle of boiling, condensation, and decantation is its primary purpose.

Vaporization (Boiling Phase): Water and chopped plant material, like *O. gratissimum* leaves, are placed in a flask with a circular bottom and brought to a boil. The components of the essential oil and water evaporate together due to the heat. This process occurs slightly below 100°C because steam prevents essential oils from mixing with water and boiling at a lower temperature. This reduces the oil's exposure to heat (Zist Yar Sanat Iranian, 2024).

Condensation: Water and essential oil are among the combined vapors that ascend into the condenser. The vapors are cooled by the cold jacket that surrounds the condenser, transforming them back into liquid droplets called distillate.

Decantation and Co-hobation (Separation Phase): The condensed liquid enters the trap, also known as the graded collector tube. This is where the Clevenger equipment performs its primary purpose. *Ocimum gratissimum* essential oil floats to the top of the aqueous distillate and forms a distinct layer because it is often lighter than water (ResearchGate, 2023). Through a diagonal return tube, the heavier lower layer, the essential oilus distillate (hydrosol), is continually recirculated back to the boiling flask.

By enabling the water to extract additional oil from the plant while retaining the separated oil, this recirculation known as co-hobation improves efficiency (Nayak 2025). Calculating the percentage yield of the plant material requires the direct measurement of the oil amount extracted, which is made possible by the collector's graduated side-arm.

## **1.2 Chemical And Physical Properties Of Essential Oils**

The physical and chemical characteristics of essential oils are crucial for both practical application and quality assurance.

Physical characteristics

1. Color: Shows tiny pigment impurities or particular phenolic chemicals, ranging from colorless to vivid yellow or green (Yadav 2022).
2. Odor: The ratio of heavier sesquiterpenes to high-volatility monoterpenes determines the intensity

and profile (Srivastava and Mathur 2022).

3. Density: Most oils are lighter than water, usually less than 1 g/cm<sup>3</sup>.
4. Refractive Index: Determines how light bends; typically ranges from 1.45 to 1.50 depending on the composition.
5. Optical Rotation: Helps detect adulteration by revealing the chirality of molecules.
6. Solubility: Not soluble in water but soluble in organic solvents and alcohol.
7. Flash Point: Typically between 50 and 70°C; crucial for handling and transportation safety.
8. Specific Gravity: The refractive index and optical rotation serve as indicators of cleanliness; most oils have refractive indices between 1.460 and 1.490, making them lighter than water (0.85–0.98 g/cm<sup>3</sup>) (Yadav 2022).

#### Chemical characteristics

1. Chemical Composition: Terpenes, terpenoids, phenylpropanoids, and aliphatic chemicals make up the majority of essential oils.
2. Volatility: At room temperature, essential oils readily evaporate due to their high volatility.
3. Boiling Point: Their boiling points, which range from 150 to 300°C, are low.
4. Reactivity: Essential oils, particularly those with functional groups like phenols and aldehydes that support antioxidant and antibacterial activity, are reactive.

Isomeric terpenoids and phenylpropanoids are distinguished by functional group analysis using FTIR and chromatographic fingerprinting (GC-MS). This procedure aids in detecting adulteration and ensuring chemotype consistency (Yadav 2022).

### 1.3 Uses Of Essential Oils

Because of their sensory and bioactive qualities, essential oils are used in many different industries.

1. Aromatherapy improves mood, protects the nervous system, and has relaxing effects by inhalation

or topical treatment (Bolouri 2022).

2. In cosmetics and perfumery, essential oils provide complex, natural aromas for air fresheners, soaps, and perfumes while masking offensive base ingredient odors. They are useful active ingredients in skincare products for a variety of advantages due to their chemical characteristics (MDPI, 2020).

3. To extend shelf life, the food and beverage industries use oils as natural flavorings and antimicrobials (Bolouri 2022).

4. Essential oils serve as fumigants, nematocides, and biopesticides in agriculture, offering environmentally benign substitutes for synthetic chemicals (Bolouri 2022).

5. Inhaling essential oils, particularly citrus and lavender oils, can promote relaxation and temporarily lessen tension (MDPI, 2023).

6. In the pharmaceutical industry, they are active components of expectorants, antiseptics, topical pain remedies (wintergreen), and cough syrups (eucalyptus) (Lizarraga-Valderrama, 2021).

7. Essential oil vapors, such as blends of clove, cinnamon, and oregano, are being investigated for air sanitation in both everyday and medical settings. Microbial and mold/yeast counts are significantly reduced in these fumes (ResearchGate, 2021).

#### **1.4 Medicinal/Pharmacological Properties Of Essential Oils**

The medicinal potential of essential oils is highlighted by a body of in vitro and in vivo research:

1. Antimicrobial action: It has broad-spectrum efficacy against viruses (eucalyptus), fungi (clove oil, cinnamon oil), and bacteria (tea tree, thyme). This happens as a result of efflux pump inhibition and membrane rupture (de Sousa 2023).

2. Anti-inflammatory/analgesic effect: Arthritis, dermatitis, and neuroinflammation can be relieved by modifying cytokine release and cyclooxygenase pathways (Nazir and Gangoo 2022). For instance, clove oil, peppermint oil, chamomile, and ginger work well.

3. Antioxidant effect: Flavonoids and phenolic terpenoids (eugenol, thymol) scavenge free radicals to lessen oxidative stress in neurological and cardiovascular disorders (de Sousa 2023).
4. Anticancer effect: Tumor cell lines show selectivity for malignant cells over normal ones by inducing apoptosis and cell cycle arrest (de Sousa 2023). For example, elements like thymol and limonene (found in citrus oil) are implicated.
5. Neuroprotective/Anxiolytic effect: These drugs help with stress, sleeplessness, and cognitive impairments by interacting with neurotransmitter receptors (serotonin, GABA) (Nazir and Gangoo 2022). Bergamot and lavender oil are two examples.
6. Insecticidal, Larvicidal, Repellent, and Acaricidal Activities: These substances function as neurotoxins that inhibit insect growth and development or interfere with respiration. They can impair the integrity of the cuticle in acaricides, kill insect larvae, and form barriers that prevent arthropods from landing or feeding. Lemon, eucalyptus, peppermint oil, and fragrance leaves, for instance, work well.

### **1.5 Plant Materials With Essential Oil**

Key botanical families and representative oil-yielding species include:

- Lamiaceae: *Mentha* spp., *Ocimum* spp., *Rosmarinus officinalis*
- Rutaceae: *Citrus* spp. (lemon, orange, bergamot, grapefruit)
- Myrtaceae: *Eucalyptus globulus* (Eucalyptus), *Melaleuca alternifolia* (tea tree), *Syzygium aromaticum* (Clove)
- Apiaceae: *Foeniculum vulgare* (fennel), *Daucus carota* (carrot seed)
- Zingiberaceae: *Zingiber officinale* (ginger), *Curcuma longa* (turmeric), *Elettaria cardamomum* (Cardamom)
- Pinaceae: *Pinus* spp (pine), *Abies* spp (fir), *Picea* spp (Spruce)
- Asteraceae: *Matricaria chamomile* (Chamomile), *Arnica montana* (Arnica)

- Liliaceae: *Allium* spp (garlic, onion)
- Lauraceae: *Cinnamomum* spp, *Laurus nobilis* (bay laurel from leaves)
- Cupressaceae: *Cupressus sempervirens* (Cypress), *Juniperus communis* (Juniper)

Variability in oil yield and composition arises from organ harvested (leaf, flower, fruit), seasonality, gEssential oilgraphical origin and post-harvest processing (Srivastava and Mathur 2022).

### **1.6 *Ocimum gratissimum* Plant**

From a systematic perspective, *Ocimum gratissimum* is classified as follows:

Kingdom; Plantae

Order; Lamiales

Family; Lamiaceae

Genus; *Ocimum*

Species; *gratissimum* L.

The genus name of *Ocimum gratissimum* L. (Lamiaceae), often known as African basil or scent leaf, comes from the Greek word "ózo, which meaning "to smell." Traditional Asian and African medicine and food make extensive use of this fragrant herb (Ashokkumar 2020). It is indigenous to South Asia, Africa, Madagascar, and the Bismarck Archipelago. Additionally, Polynesia, the Caribbean, Central America, and portions of South America have received it. Essential oil has historically been applied topically to treat skin problems and used as a fumigant or repellent for home pests. *O. gratissimum*'s essential oils, which are rich in phenolic compounds like thymol and eugenol, are primarily responsible for the plant's efficacy in medicine and as a biocide.

These substances are well-known for having potent insecticidal and antibacterial properties (Ayinde 2023).

The shrub *Ocimum gratissimum* L. can reach heights of 0.5 to 3 meters. It has opposing, ovate-lancers and square stems. Essential oillate leaves are glandular-pubescent on the underside and are

3.5–16 cm by 1–8.5 cm. Small, bilabiate, white-greenish blooms are produced by its verticillaster inflorescences. This plant yields between 0.5 and 3.5% essential oil, with high concentrations of eugenol and thymol. The best conditions for cultivation are full sun, tropical to subtropical temperatures, and well-drained, loamy soil. Uniform chemotypes appropriate for commercial oil production are produced by propagation using seeds or stem cuttings.

To obtain the maximum oil yield, which varies based on the chemotype and environmental conditions, leaves are usually removed during peak flowering. The plant is a sustainable option for agroecological systems since it is frequently planted with other herbs or vegetables and is valued for its pest-repelling properties (Plantary 2023).

### **1.7 Traditional Uses Of *Ocimum gratissimum***

*Ocimum gratissimum* L. has long been used in traditional cookery, healing, and cultural customs. *O. gratissimum* infusions and decoctions are used in West African ethnomedicine to treat respiratory conditions such as bronchitis, asthma, cough, and catarrh. Because of its antispasmodic and antisecretory properties, leaf extracts help treat gastrointestinal issues such as diarrhea, dysentery, flatulence, and stomach pains. To cure malaria symptoms and lower fever, boiled leaves are eaten or inhaled as steam. Ringworm and boils are treated by applying crushed leaves directly to the skin.

This plant is used as a parturient aid, assists with childbirth, controls menstruation, and heals vaginal infections in some groups. It helps with digestion, acts as a natural food preservative, and is frequently used as a seasoning in soups and sauces. Deep cultural linkages are reflected in its employment in rituals for spiritual protection, funerals, and wound cleaning (Ugbogu 2021).

### **1.8 Reported Pharmacological Properties Of *Ocimum gratissimum***

1. Antimicrobial property: *Ocimum gratissimum* Essential oil and its derivatives exhibit potent inhibitory actions against a variety of strains, including many fungi and both Gram-positive and

Gram-negative bacteria (NIH, 2021). This is because the lipophilic properties of eugenol and thymol cause the microbial cell membrane to rupture, resulting in leaking and cell death (ResearchGate, 2021).

2. Anti-inflammatory/Analgesic property: *O. gratissimum*'s primary ingredient, eugenol, has been shown to suppress pro-inflammatory mediators including TNF- $\alpha$ , IL-6, and COX-2, confirming the plant's anti-inflammatory benefits (Ashokkumar 2021). This validates its historical application in treating pain and fever.

3. Antioxidant activity: In tests such as DPPH and ABTS, *Ocimum gratissimum* Essential oil regularly demonstrates a high antioxidant capacity, which is directly related to the content of phenolic compounds (JABS, 2023; MDPI, 2024). Food preservation and nutritional supplements exploit this scavenging function, which is essential for lowering oxidative damage (MDPI, 2024).

4. Antidiarrheal property: Through antispasmodic and antisecretory properties, essential oil leaf decoctions lessen castor oil-induced diarrhea in rats (Ugbogu 2021).

5. Antidiabetic/Antihypertensive activity: Although more specific mechanisms must be elucidated, the plant extracts exhibit promise in regulating blood glucose levels and enhancing heart health (NIH, 2021).

6. Anticancer and cytotoxic Activity: In a number of cancer cell lines, including models of breast and colon cancer, eugenol has been demonstrated to induce apoptosis and stop the cell cycle. DNA fragmentation, caspase activation, and mitochondrial malfunction are suggested to be the causes of this (Imosemi, 2020).

7. Anxiolytic activity: In animal experiments, *Ocimum gratissimum* hydroethanolic and essential oil extracts have demonstrated anti-stress and anxiety-reducing properties. This activity is related to the plant's capacity to affect stress-associated alterations in biochemical markers and membrane dynamics and is comparable to conventional anti-anxiety drugs (ResearchGate, 2024).

8. Neuroprotective: *Ocimum gratissimum* essential oil helps prevent neurodegenerative illnesses by

lowering oxidative stress in the brain (MDPI, 2025). It is believed that its rich phytochemical composition, which includes flavonoids and phenolic substances, supports cognitive function and preserves nerve health (ResearchGate, 2024).

9. Anti-Anaemic Activity: Following chemically induced anemia, *Ocimum gratissimum* leaf extracts can improve blood profiles by markedly raising hemoglobin (Hb), packed cell volume (PCV), and red blood cell (RBC) counts (Akara 2021).

10. Wound healing properties: In animal models, *Ocimum gratissimum* extracts significantly improve wound healing, as evidenced by a shorter epithelialization period and faster wound contraction rates (ResearchGate, 2025).

11. Anti-fertility Effects: According to traditional methods and some earlier research, the herb has anti-fertility effects on males (ResearchGate, 2024).

12. Antinociceptive Activity: Studies using rat models validate this essential oil's capacity to reduce nociceptive and neuropathic pain. Although there are some small toxicity concerns, its effects are comparable to those of synthetic painkillers. The primary substances involved in pain alleviation are myrcene and eugenol (Ajayi 2021).

13. Cytoprotective Roles: The plant's antioxidant qualities are connected to its historic use as a tonic, indicating that it guards against oxidative stress-related illnesses such liver damage and several cardiac disorders (NIH, 2021).



**Figure 1: Picture of *Ocimum gratissimum* leaf**

## 1.9 Overview Of Ticks And Tick-Borne Diseases

### Ticks and Tick-Borne Diseases in West Africa

Ticks flourish in hot, humid climates in tropical places, particularly in Sub-Saharan Africa (SSA). Public health and cattle productivity continue to face difficulties as a result (Evans 2024; Bastos et al., 2019). Worldwide, more than 800 tick species live in a variety of settings. For each species to develop through the egg, larval, nymphal, and adult phases, a blood meal is required at various times of life (Temple and Taylor, 2023). Ticks release a complex mixture of immune-modulating, anti-inflammatory, and anticoagulant substances when they attach. These characteristics enable them to feed for extended periods of time and raise the possibility that they will infect their hosts with viruses (Temple and Taylor, 2023).

Together, there are around 900 species of hard ticks (Ixodidae) and soft ticks (Argasidae). There are over 700 kinds of hard ticks and 193 species of soft ticks (Cuffari, 2023). Ixodid ticks typically feed once during each stage of development. In order to cling to passing hosts, they search for vegetation. On the other hand, argasid ticks typically live in burrows or nests and eat sporadically (Cuffari, 2023). The majority of ticks have several hosts during their life cycle. This makes it more difficult to regulate them and increases their capacity to spread different bacteria, viruses, protozoa, and poisons (Cuffari, 2023). *Ixodes*, *Rhipicephalus*, *Amblyomma*, and *Dermacentor* are among the genera of ixodid ticks that are extremely important in the medical and veterinary domains. Lyme borreliosis, babesiosis, anaplasmosis, Rocky Mountain spotted fever, and rickettsioses are among the illnesses they spread. *Theileria parva* (East Coast fever) and *Ehrlichia ruminantium* (heartwater) are carried by *Rhipicephalus* and *Amblyomma* species in Sub-Saharan Africa. In cattle and small ruminants, many illnesses cause serious illness and even death. Unusual clinical symptoms and co-infections complicate diagnosis and therapy, underscoring the necessity of comprehensive surveillance. Synthetic acaricides such as amidines, pyrethroids, and organophosphates are used in traditional control approaches. However, these compounds' long-term efficacy is limited by environmental

residue problems and chemical resistance. This circumstance emphasizes the necessity of investigating sustainable and alternative control strategies, such as plant-based acaricides with different modes of action (CDC, 2024; Temple and Taylor, 2023).

## **1.10 Justification Of The Study**

Three primary factors make this research significant: the potential of local plants, health and environmental issues, and acaricide resistance.

### **1.10.1 Control Failure and Acaricide Resistance**

Tick populations throughout Africa have rapidly developed multi-drug resistance as a result of the extensive and frequently negligent usage of synthetic acaricides like organophosphates and pyrethroids. Many traditional treatments become unsuccessful as a result (Githae 2021). Resistance increases the expense of managing animals and causes major control failures on farms. Farmers frequently use costly, inefficient treatments or high-dose sprays.

### **1.10.2 Environmental and Public Health Concerns**

1. Acaricide Resistance Crisis: Ticks have become resistant to almost all synthetic chemicals, particularly dangerous species like *Rhipicephalus microplus* (Reshma and Prakasan, 2025). Farmers frequently use bigger quantities than advised, increase application frequency, or rotate chemicals inappropriately in response to treatment failures. Resistance develops more quickly as a result (Frontiers, 2025).
2. Contamination and Residues: Older synthetic acaricides, such as organochlorines, are difficult to decompose and build up as hazardous residues in soil, water, and food items like milk and meat (Frontiers, 2025).
3. Risk to Human Health: Acaricides are frequently handled improperly in poor nations. Farm workers may have health problems such headaches, lightheadedness, and skin irritation as a result (PMC, 2025).

### **1.10.3 Tapping into Ethnobotanical Potential**

1. *Ocimum gratissimum* is a well-known plant whose potent anti-parasitic and insecticidal qualities were discovered through ethnobotanical surveys and laboratory testing (ResearchGate, 2025).

2. New Modes of Action (MoA): A variety of chemicals found in botanical acaricides target ticks via a variety of interrelated processes (ResearchGate, 2025). Compared to single-molecule synthetic medications, this makes it more difficult for ticks to build resistance (MDPI, 2024).

This research will support the increasing demand for environmentally friendly and biodegradable products that can fit into Integrated Tick Management (ITM) programs, particularly in areas where the plant is native and available, by demonstrating the acaricidal effectiveness, characterizing the local chemotype, and elucidating the mechanism of action of *Ocimum gratissimum* volatile oil (FAO, 2025).

### **1.11 Objectives Of The Study**

The goals of this study include:

1. To extract *Ocimum gratissimum* volatile oil and evaluate its yield.
2. To assess and measure the in vitro acaricidal activity of *Ocimum gratissimum* volatile oil against cattle ticks.
3. To compare the effectiveness of the *Ocimum gratissimum* treatment to a control group.
4. To discuss the implications for developing plant-based acaricide formulations and provide suggestions for further research, including safety, formulation, and field trials.

## CHAPTER 2

### MATERIALS AND METHODOLOGY

#### 2.1 Equipment And Apparatus

These include heating mantle, micro pipettes (20 $\mu$ L), test tubes and test tube racks, refrigerator, stirrer, spatula, digital weighing balance, round-bottom flask, Petri dishes, measuring cylinder, beaker, Clevenger type apparatus, conical flask, column tube, TLC plate, TLC tank, oven, spraying bottles, capillaries, sample bottle, mortar and pestle.

#### 2.1.2 Reagents and Chemicals

Acetone, hexane, ethyl acetate, 12% sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), silica gel.

#### 2.1.3 Consumables

Marker, tape, cotton wool, filter paper.

#### 2.2 Collection and Preparation of Plant Material

Fresh, healthy leaves of *Ocimum gratissimum* L. were bought from local markets in Benin City.

#### 2.3 Extraction of Essential Oils from the Plant

Oil from *O. gratissimum* leaves was extracted by steam distillation using a Clevenger-type apparatus, and 2.49 kg of the plant material was put into a round-bottom flask. The flask was heated using the heating mantle after enough water had been added to cover half of the plant material. The oil condensed in the condenser and flowed into the apparatus's receiving arm as the water boiled and evaporated. Because the extracted oil is less dense than water, the volume was determined using the calibration on the receiving arm. The volatile oil was gathered in sample vials and kept at 4 °C in a refrigerator.

#### 2.4 Determination of Oil Yield

The volume obtained was divided by the weight, and the result was multiplied by 100% to determine the percentage yield of the plant material (0.6%). The amount of oil produced was recorded.

## **2.5 Fractionation and Isolation using Column Chromatography (Cc)**

To separate the active fractions from the oil, chromatographic separation was performed. Since this technique efficiently separates medium-polarity molecules typical of essential oils and plant phenolics, the stationary phase and column were created by combining silica gel as a slurry in the mobile phase solvent. To prevent air bubbles, which are essential for obtaining excellent resolution, the column was packed using the wet-packing technique. A tiny amount of silica gel was adsorbed with a predetermined volume of the active volatile oil (12 ml of *Ocimum gratissimum* volatile oil). Carefully, the sample was positioned on top of the densely packed stationary phase.

In order to select the mobile phase, the polarity of the mobile phase was gradually increased using a step-gradient elution approach. Using 100% hexane (test tubes 1–22), hexane-ethyl acetate combinations 99:1 (test tubes 23–45), 98:2 (test tubes 46–68), and 97:3 (test tubes 69–100), this procedure guaranteed the separation of a broad variety of chemicals. TLC was used to monitor all obtained fractions using a Hexane-ethyl acetate (9.5:0.5) solvent solution. 36 fractions were obtained by spraying the TLC plates with a general visualizing agent, such as 12% H<sub>2</sub>SO<sub>4</sub>, and heating them in the oven at 114 °C for five minutes. Two significant sub-fractions (F32-F50, F51-F68) were obtained by combining fractions with comparable values and visualization patterns.

## **2.6 Collection and Identification of Tick Species**

With the help of Fulani cattle herders, ticks were extracted from livestock at the cattle ranch near Benin Federal Girls College Road and Upper Mission Road in Benin City. The ectoparasites were kept in aerated plastic containers after being manually removed from the cattle's skin. The ticks were categorized into various species using the Tick Atlas. There were two species found.

## **2.7 Preparation and Administration of Different Concentrations of the Volatile Oil**

The volatile oil collected from the plant was diluted into different quantities of 1%, 2%, 5%, and

10% using acetone. For instance, to make a 10% oil concentration in 10 ml, 1 ml of the volatile oil was combined with 9 ml of acetone. Because acetone is inert and quickly evaporates, it was selected as the solvent. A graduated micro pipette was used to apply 20  $\mu$ L of each concentration topically to ten ticks of the same species that were placed in a petri dish. After that, the outcomes were tracked. 20  $\mu$ L of acetone were used as a control. Over the course of twenty four hours, the mortality rate and the amount of time it took for each tick to become completely motionless were noted. The same procedure was carried out in triplicate for other tick species that were available.

## CHAPTER THREE

### RESULTS

#### 3.0 Percentage Yield

2.49 kg of fresh *O. gratissimum* leaves yielded 15 mL of volatile oil, or 0.6%, at the conclusion of the steam distillation of the volatile oils using a Clevenger-type apparatus. This is regarded as a moderate return and falls within the expected range for essential oils from the fresh leaves of *Ocimum* species.

#### 3.1 Identities of the Ticks

By comparing them with images of the species obtained from the Tick Atlas, the identities of the two tick species utilized were verified. These species are *Hyalomma rufipes* and *Rhipicephalus microplus*. The most prevalent species in the population was discovered to be *R. microplus*.

#### 3.2 Percentage Mortality Rate of *O. gratissimum* on *Hyalomma rufipes* and *Rhipicephalus microplus*

The volatile oil's effectiveness as a deadly agent against the tick genus *Hyalomma rufipes* was confirmed when it reached 100% mortality at 10% concentration. The oil maintained a high mean mortality of  $86.67 \pm 15.28$  even at the lowest concentration tested (1%). Mortality rises with concentration, from 86.67% at 1% to 100% at 10%. The low standard deviation at 5% and 10% concentrations (0.0 and 5.77, respectively) indicates that the cidal impact is concentration dependent and increases with concentration. The Acetone control group exhibits 0% mortality, indicating that the toxicity of the *O. gratissimum* volatile oil was the only cause of the tick fatalities that were detected.

At concentrations of 2%, 5%, and 10%, the oil completely killed *Rhipicephalus microplus* in every

replicate. Perfect reliability is indicated by the higher doses' zero standard deviation. A remarkable mean mortality of  $93.3 \pm 11.55$  was obtained even at the lowest concentration tested (1%). The observed tick fatalities were exclusively caused by the volatile oil, as evidenced by the 0% mortality in the acetone control.

### **3.3 Percentage Mortality of Fraction 1 and Fraction 2 (1% Concentration) on Two Tick Species**

According to the results, Fraction 2 had a mortality rate of  $100 \pm 0$  for *Rhipicephalus microplus*, while Fraction 1 had a mortality rate of  $86.7 \pm 11.55$ . Fraction 1's mortality rate for *Hyalomma rufipes* was  $100 \pm 0$ , marginally better than F2's  $96.7 \pm 5.77$ .

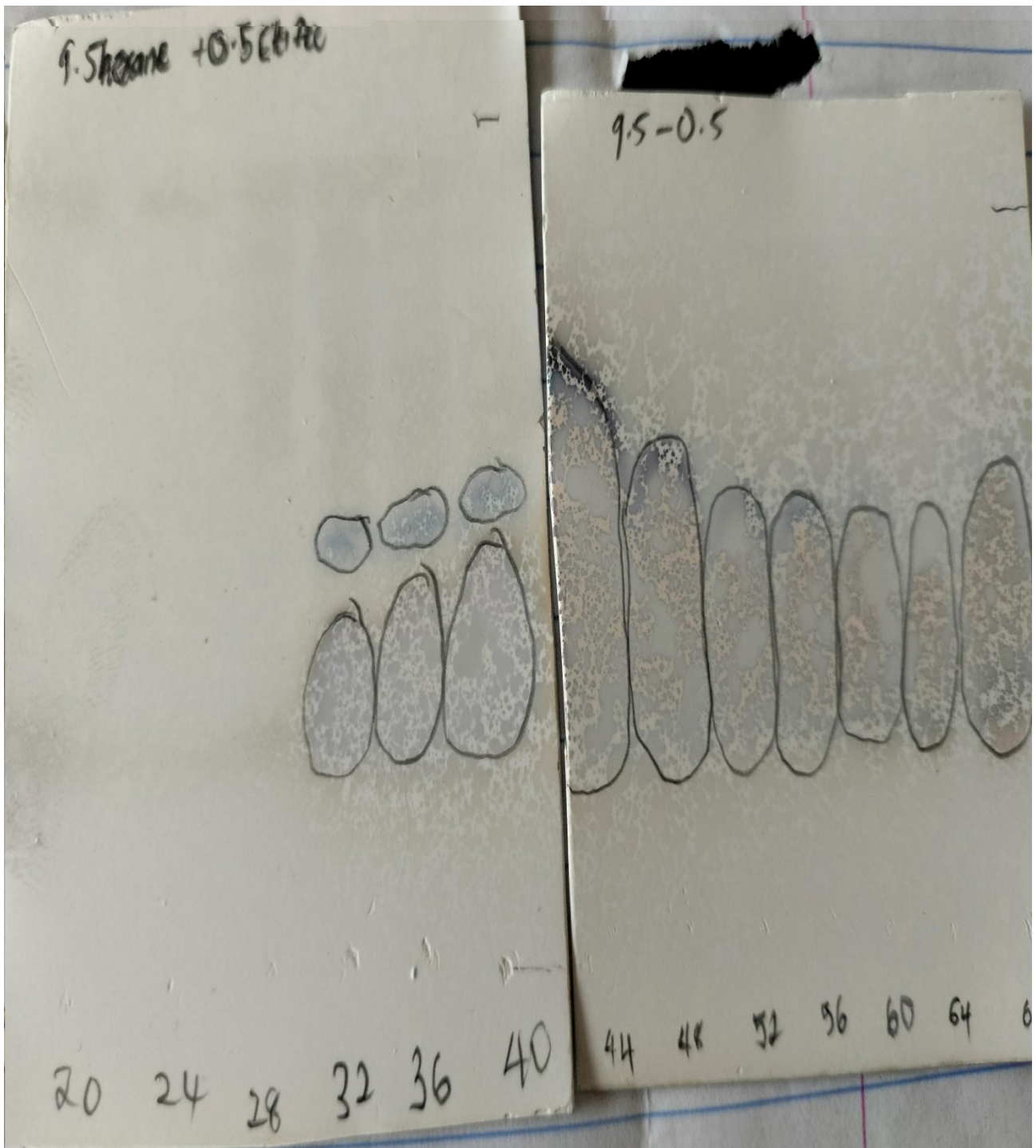


Figure 2; TLC plate monitoring *Ocimum gratissimum* volatile oil fractions

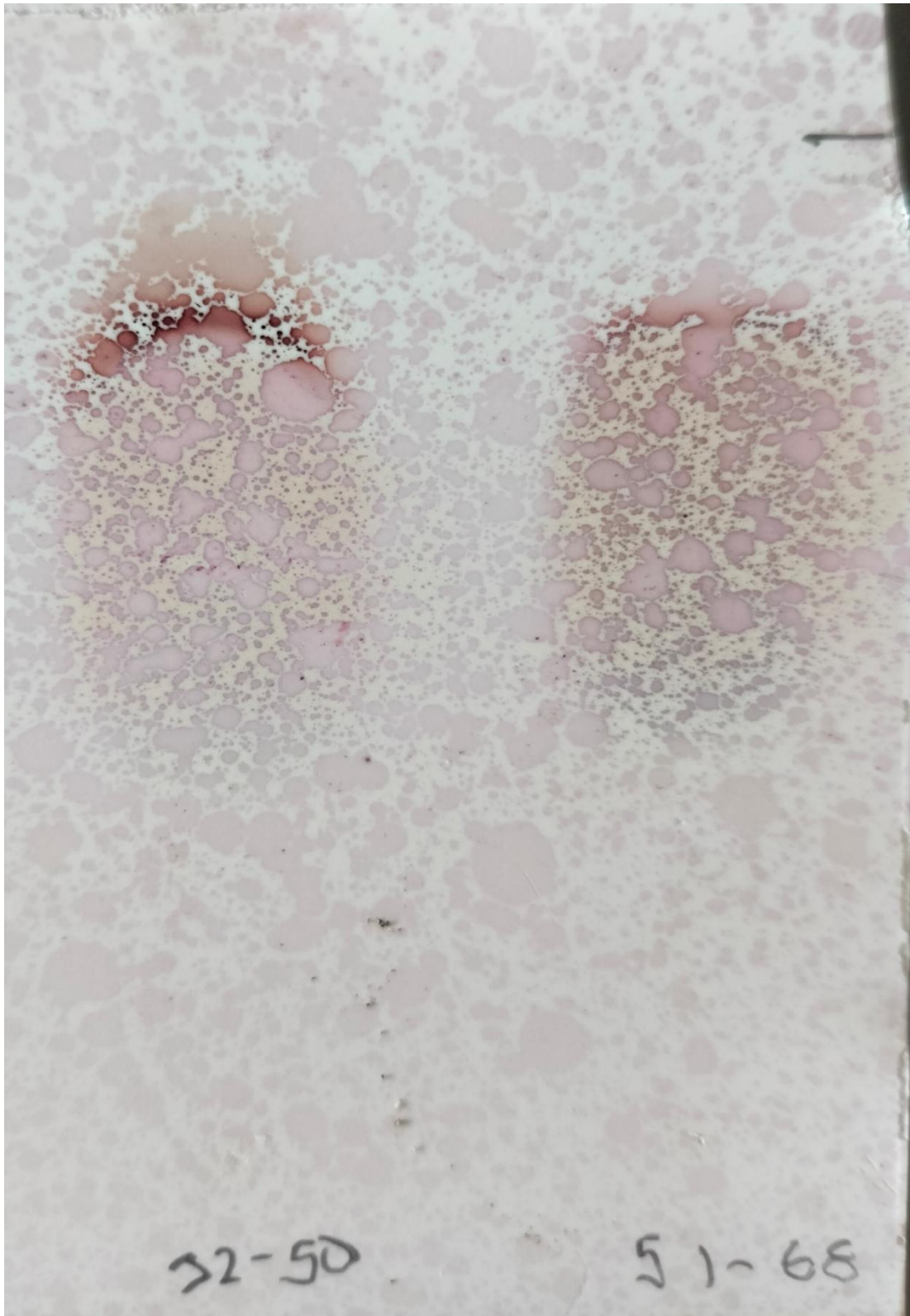


Figure 3; TLC plate monitoring *Ocimum gratissimum* volatile oil of the combined fractions

**Table 1; Percentage Mortality Rate of *O. gratissimum* on *Hyalomma rufipes* and *Rhipicephalus microplus***

Concentrations (%)	% Mortality	
	<i>Hyalomma rufipes</i>	<i>Rhipicephalus microplus</i>
1	86.67±15.28	93.3±11.55
2	90.0±10.00	100±0
5	96.67±5.77	100±0
10	100±0	100±0
Acetone	0	0

**Table 2; Percentage Mortality of Fraction 1 and Fraction 2 (1% Concentration) on Two Tick Species**

Concentrations (%)	% Mortality	
	<i>Hyalomma rufipes</i>	<i>Rhipicephalus microplus</i>
Fraction 1 (32-50)	100±0	86.7±11.55
Fraction 2 (51-68)	96.7±5.77	100±0
Acetone	0	0

## CHAPTER FOUR

### DISCUSSION

Studying plants as acaricidal agents is crucial because it addresses major flaws in the way ticks are currently managed, particularly with regard to the negative effects of synthetic acaricides. This study directly addresses the need for novel chemicals on a global scale. Ticks, especially *R. microplus*, have developed widespread multi-drug resistance due to the widespread use of conventional pesticides like pyrethroids and organophosphates. The varied, multi-target mechanisms of action provided by botanical substances make it more difficult for the parasite to build resistance. The prevalent, unsustainable resistance of *R. microplus* to synthetic chemicals (pyrethroids, organophosphates) is addressed by an effective natural product (Reshma and Prakasan 2025; MDPI 2024; ResearchGate 2025). The product is anticipated to decompose rapidly due to its volatile nature, reducing the risk to the public's health from hazardous chemical residues in meat, milk, and the environment (PMC 2025; Reshma and Prakasan 2025). The current reliance on synthetic chemical control has significant drawbacks, such as toxic residues, which raise concerns about food safety and public health because synthetic chemicals may leave hazardous, persistent residues in the environment and in animal products (meat and milk) (Onwudiegwu 2025; Reshma 2020). User safety is also an issue because agricultural workers may handle and apply synthetic chemicals improperly owing to a lack of resources or training, which could result in negative health impacts such as skin irritation and neurotoxic symptoms (Gichure 2025; Onwudiegwu 2025). This study demonstrates a natural, quick-acting solution based on readily available ethnobotanical materials, supporting the need for an environmentally benign, biodegradable substitute. Because the active components of *Ocimum gratissimum* (OG) volatile oil are often semi-volatile terpenoids and phenolics, the successful separation and biological screening of OG volatile oil components is crucial. Its potential as a robust botanical acaricide is confirmed by isolation, which demonstrates excellent efficacy against two commercially significant tick species: *Rhipicephalus microplus* and *Hyalomma*

*rufipes*. This approach consistently recovers solvent-free volatile chemicals, as demonstrated by the successful isolation using the Clevenger-type apparatus. Particularly against *Rhipicephalus microplus*, the most common tick species in the sample, the crude volatile oil shown exceptional efficacy. The extensive resistance of *Rhipicephalus microplus* to commercial acaricides is well known, and the high mortality rate suggests that the oil may be more effective than many synthetic compounds. *Hyalomma rufipes* also showed substantial mortality, demonstrating the *O. gratissimum* oil's widespread efficacy against a variety of tick genera that are known to spread illness to both humans and animals. Strong activity from hydrocarbon monoterpenes or early oxygenated monoterpenes is suggested by the mortality shown in Fraction F1, although highly active phenols like thymol and eugenol, which demonstrated exceptional efficacy, particularly against *R. microplus*, are probably present in Fraction F2. These results suggest that several compounds contribute to the acaricidal effect. Both F1 and F2's consistent high efficacy clearly points to a combined action, in which the oil as a whole is more potent than its component parts. When opposed to single-molecule synthetics, plant pesticides have this major benefit. The fact that both fractions continued to be very effective against all three species at low concentrations highlights the potency of the phytochemical mixture as a whole as a source of acaricidal substances. The primary mechanisms of action suggested are membrane disruption and neurotoxicity. According to research, *O. gratissimum* volatile oil has a high concentration of phenolic chemicals, including thymol and eugenol, which are arthropod poisons that have an impact on the neurological system. They function by acting as antagonists on non-cholinergic locations, such as octopamine receptors, or by blocking vital invertebrate-specific enzymes like acetylcholinesterase (AChE). Overstimulation, paralysis, and quick death may result from these behaviors (Oliveira 2022). Since the essential oil is greasy, it can pass through the tick's chitinous cuticle and damage the integrity of the cell membranes in the respiratory and neurological systems, impairing cellular function and respiration (Oliveira 2022).

## CHAPTER FIVE

### CONCLUSION

The efficacy of *Ocimum gratissimum* volatile oil against significant cow ticks was investigated in this study. The plant's promise as a natural biopesticide for tick control is confirmed by the results.

At concentrations as low as 2%, the volatile oil demonstrated exceptional efficacy, achieving 100% mortality against the cattle tick, *Rhipicephalus microplus*.

The fact that fractions 1 and 2 both achieved 100% mortality against *Hyalomma rufipes* and *Rhipicephalus microplus*, respectively, suggests that their effects are synergistic.

In conclusion, *Ocimum gratissimum* volatile oil is a particularly good option for developing a long-lasting, efficient, and eco-friendly integrated tick management product.

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## APPENDIX

**Table 3: Percentage Mortality Rate of *O. gratissimum* on *Hyalomma rufipes***

% Concentration	Group A	Group B	Group C	Mean	Standard Deviation (SD)	Mean $\pm$ SD
1%	90	90	100	93.3	5.77	93.3 $\pm$ 5.77
2%	70	60	100	76.7	20.82	76.7 $\pm$ 20.82
5%	80	100	100	93.3	11.55	93.3 $\pm$ 11.55
10%	100	100	100	100	0	100 $\pm$ 0
Acetone %	0	0	0	0	0	0

**Table 4: Percentage Mortality Rate of *O. gratissimum* on *Rhipicephalus microplus***

% Concentration	Group A	Group B	Group C	Mean	Standard Deviation (SD)	Mean ± SD
1%	80	100	100	93.3	11.55	93.3±11.55
2%	100	100	100	100	0	100±0
5%	100	100	100	100	0	100±0
10%	100	100	100	100	0	100±0
Acetone %	0	0	0	0	0	0

**Table 5: Percentage Mortality of Fraction 1 (1% Concentration) on Two Tick Species**

Tick species	Group A	Group B	Group C	Mean	Standard deviation (sd)	Mean $\pm$ sd
<i>Rhipicephalus microplus</i>	80	80	100	86.7	11.55	86.7 $\pm$ 8.67
<i>Hyalomma rufipes</i>	100	100	100	100	0	100 $\pm$ 0

**Table 6: Percentage Mortality of Fraction 2 (1% Concentration) on Two Tick Species**

Tick species	Group A	Group B	Group C	Mean	Standard deviation (sd)	Mean ± sd
<i>Rhipicephalus microplus</i>	100	100	100	100	0	100±0
<i>Hyalomma rufipes</i>	90	100	100	96.7	5.77	100±5.77