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Faculty of Science

Biological Sciences Department

Evaluation of the Potential Efficacy of *Lipia javanica* (Burm.f.) Spreng. (Zumbani) Crude Leaf Extracts against *Rhipicephalus appendiculatus* (Neumann, 1901) Tick Larvae.

By

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APPROVAL FORM

Dissertation Tittle: Evaluation of the Potential Efficacy of *Lipia javanica* (Burm.f.) Spreng. (Zumbani) Crude Leaf Extracts against *Rhipicephalus appendiculatus* (Neumann, 1901) Tick Larvae.

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The undersigned confirm that they have reviewed the dissertation and consider it appropriate for submission to the Faculty of Science. They have also verified its compliance with the faculty's guidelines.

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DECLARATION

I, Munoda Flame Tapfuma, affirm that the herein presented research is my original work and that any sources or contributions from others have been properly acknowledged, whether through electronic means or in other forms.

SIGNATURE: 

DEDICATION

I dedicate this project to the Living Beyond Hope family for their unwavering support throughout my academic journey.

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LIST OF ABBREVIATIONS

AChE: Acetyl cholinesterase

ANOVA: Analysis Of Variance

DNA: Deoxy-ribose Nucleic Acid

GABA: Gamma-Amino Butyric Acid

LC_{50}: Lethal Concentration 50

ROS: Reactive Oxygen Species

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ABSTRACT

Ticks are a major challenge to livestock production in sub-Saharan Africa due to their role in transmitting diseases such as East Coast fever caused by *Theileria parva*. Conventional control mainly depends on chemical acaricides; however, their continued use has led to the development of acaricide resistance, environmental concerns, and high costs. These limitations highlight the need for alternative and sustainable tick control methods. Therefore, this study evaluated the acaricidal activity of *Lipia javanica* (Burm.f.) Spreng crude leaf extracts against the larvae of *Rhipicephalus appendiculatus*, the principal vector of *Theileria parva*. *Lipia javanica* leaves were collected, air-dried, ground, and macerated in 70% ethanol and distilled water as extraction solvents. The resulting crude extracts were tested for acaricidal activity at concentrations of 30%, 50%, and 100% using a modified larval packet test. Amitraz (0.03%) and distilled water were used as positive and negative controls, respectively. Larval mortality was assessed after 48 hours of incubation under controlled laboratory conditions. The results showed a concentration-dependent increase in mortality for both extraction solvents, although ethanol extracts demonstrated greater acaricidal activity than distilled water extracts. The ethanol extracts produced mean larval mortalities of 13, 23, and 33.2 dead larvae at the 30%, 50%, and 100% extract concentrations, respectively. One-way ANOVA for the ethanol extracts showed significant differences among treatments and controls ($F = 1143.64$, $p < 0.001$). Bonferroni post hoc pairwise comparisons showed that each extract concentration (30%, 50%, and 100%) differed significantly from both the positive and negative controls ($p < 0.001$). The analysis also showed significant differences between the 30% and 50% concentrations and between the 30% and 100% concentrations ($p < 0.001$) for the ethanol extracts. However, no significant difference was observed between the 50% and 100% concentrations ($p > 0.05$). The distilled water extracts resulted in lower mean larval mortalities of 2.2, 2.6, and 9.4 dead larvae for the corresponding concentrations. For the distilled water extracts, One-way ANOVA showed significant differences in larval mortality among the treatment groups ($F = 1892.98$, $p < 0.001$). Bonferroni post hoc pairwise comparisons revealed that the 30% and 50% extract concentrations did not differ significantly from the negative control ($p = 1.000$), indicating mortality levels compared to natural mortality. The 100% extract concentration differed significantly from both the positive control and negative control ($p < 0.001$). However, no significant differences were observed between the 100% concentration and the 30% and 50% concentrations ($p > 0.05$). The positive control, Amitraz, achieved complete larval mortality. However, none of the tested *Lipia javanica* leaf extract concentrations achieved 50% mortality, and the estimated LC_{50} (≈182.35%) exceeded the maximum concentration evaluated (100%), indicating relatively low acaricidal potency of the crude extract. Distilled water extracts were excluded from LC_{50} estimation because they produced insufficient mortality to establish a reliable dose–response relationship. These findings suggest that the distilled water extracts exhibited limited acaricidal activity across the tested concentrations compared with Amitraz. They indicate that the extracts of leaves of *Lipia javanica* exhibit acaricidal activity but their effectiveness is restricted when applied alone. However, their bioactivity makes them a promising tool for integrated pest management systems and complementary or alternative tick control method in resource-poor

environments. To improve the acaricidal efficacy, further research should focus on optimizing extraction methods, evaluating higher extract concentrations, developing synergistic formulations, and exploring nanotechnology-based delivery systems.

CHAPTER 1: INTRODUCTION

1.1 Background of the study

Ticks are significant ectoparasites that pose serious health risks to livestock and wildlife through transmission of different types of pathogens, including bacteria, viruses and protozoans (Norval et al., 1985). These pathogens lead to diseases such as Theileria, gall sickness, and Babesiosis, which can cause severe impacts on animal health and productivity (Kasaija et al., 2021). Therefore, controlling the population of ticks is important for the health of the infected animals. *Rhipicephalus appendiculatus*, also known as brown eared ticks, is a hard tick belonging to the Ixodidae genus, affecting livestock mainly in southern Africa. *Rhipicephalus appendiculatus* acts as a carrier of *Theileria parva*, responsible for East Coast Fever among cattle (Kasaija et al., 2021).

Conventional methods of tick control have been heavily dependent on chemical acaricides. While these chemicals are effective in reducing tick populations, their use comes with several challenges. A primary concern is linked to environmental pollution since chemical residues end up contaminating soil and water, thus posing harm to non-target organisms and ecosystem stability (de Oliveira et al., 2015). Moreover, excessive dependence on these chemicals resulted in resistance among tick populations, especially *Rhipicephalus appendiculatus* (Chitombo et al., 2021), making such treatments increasingly ineffective. Acaricides can also have negative health implications on both people and animals, posing additional problems associated with the use of these chemicals.

Due to these drawbacks, alternative approaches to chemical acaricides have been explored, including the use of medicinal plants and plant-derived extracts. One plant species with promising considerable potential is *Lipia javanica* (commonly known as Zumbani in Zimbabwean Shona), a traditional herb widely used in Southern Africa. *Lipia javanica* contains bioactive phytochemicals with demonstrated pesticidal properties, suggesting potential as a natural acaricidal agent (Maroyi, 2017). This study could be useful for sustainable tick control by investigating its acaricidal activity. Its leaves are said to have anti-microbial effects, which can be helpful to a family afflicted with an illness from the concept that brings these plants in the first place—diseases carried by ticks. It is reported that studies have indicated *Lipia javanica* has possible acaricidal properties (Madzimure et al., 2011), making it a potential natural solution for tick control.

1.2 Problem Statement

Rhipicephalus appendiculatus poses serious health risks to livestock and wildlife by transmitting *Theileria parva*, the etiological agent responsible for East Coast fever in cattle. The conventional control of ticks using chemical acaricides has, despite their effectiveness, posed negative impacts on non-target species and the environment (de Oliveira et al., 2015). Ticks have become resistant to these chemical acaricides, thus calling for new control methods (George et al., 2004). While *L. javanica* is recognised for its medicinal and insecticidal properties, its specific acaricidal efficacy against *Rhipicephalus appendiculatus* larvae,

particularly under standardised laboratory conditions to determine lethal concentration values (LC_{50}) with a focus on specific extraction methods, remains insufficiently explored.

1.3 Aim of the Study

This study aims to evaluate the acaricidal activity of *Lipia javanica* crude leaf extracts against *Rhipicephalus appendiculatus* tick larvae and to document traditional uses within a framework of integrated pest management.

1.4 Objectives

The objectives of this study are:

1. To assess the acaricidal activity of *Lipia javanica* crude leaf extracts against *Rhipicephalus appendiculatus* larvae using Amitraz as a positive control.
2. To compare the effect of different *Lipia javanica* extract concentrations (30%, 50%, and 100%) on the mortality of *Rhipicephalus appendiculatus* larvae.
3. To determine the lethal concentration 50 (LC_{50}) of *Lipia javanica* extracts against *Rhipicephalus appendiculatus* larvae.
4. To compare the effect of extraction solvent (70% ethanol versus distilled water) on the acaricidal effectiveness of *Lipia javanica* extracts against *Rhipicephalus appendiculatus* larvae.

1.5 Research Questions

The research questions are:

1. Do *Lipia javanica* crude extracts have acaricidal activity against *Rhipicephalus appendiculatus* larvae, and how does it compare to Amitraz, a known effective chemical acaricide?
2. Is there a significant difference in larval mortality of *Rhipicephalus appendiculatus* at 30%, 50% and 100% *Lipia javanica* leaf extract concentrations, and does it differ with the positive (Amitraz) and negative (distilled water) control?
3. What is the Lethal concentration 50 (LC50) of *Lipia javanica* extracts required to effectively inhibit the growth of *Rhipicephalus appendiculatus* larvae?
4. Does the extraction solvent (70% ethanol versus distilled water) influence the acaricidal effectiveness of *Lipia javanica* leaf extracts against *Rhipicephalus appendiculatus* larvae?

1.6 Hypotheses

Null Hypothesis (H0):

H0: There is no significant difference in *Rhipicephalus appendiculatus* larval mortality among the different *Lipia javanica* leaf extract concentrations (30%, 50%, and 100%), and the positive control (Amitraz) and negative (distilled water) controls,

H0: The extraction solvent (70% ethanol versus distilled water) does not influence the acaricidal effectiveness of *Lipia javanica* leaf extracts against *Rhipicephalus appendiculatus* larvae.

1.7 Significance of research

The research on *Lipia javanica* crude extracts and its effects against *Rhipicephalus appendiculatus* larvae is important both from scientific point of view. Establishing the acaricidal activity of extracts offers preliminary information on the plant's possibility as a natural tick control agent and supports traditional uses of the plant. The information offers a viable, environmentally friendly, non-chemical alternative solution for livestock farmers to control ticks that can lead to acaricide resistance and contribute to environmental contamination, as well as to reduce chemical residues in animal products at a reasonable cost. The effectiveness of the extracts can be further quantified by determining the lethal concentration (LC_{50}), which provides a measure of the concentration required to kill 50% of the exposed tick larvae. Determining LC_{50} is important because it enables assessment of extract potency, facilitates standardization of dosage levels for practical application, and allows comparison of the efficacy of *Lipia javanica* extracts with other botanical and synthetic acaricides. The results might be used in integrated pest management programs, to improve livestock health, and to encourage sustainable use of local plant resources to benefit local economies. Finally, this study helps to inform the creation of safe, effective, and locally available tick control strategies and disease mitigation measures.

CHAPTER 2: LITERATURE REVIEW

2.1 Biology of Ticks

Ticks are ectoparasitic arachnids belonging to the Acarina order (Natsuaki, 2021). They play an important role in the transmission of different pathogens to humans and animals, thus being important carriers for diseases such as Theileria, Ehrlichiosis, and Babesiosis (Norval et al., 1985). Their life cycle comprises four stages: egg, larva, nymph, and adult, with every developmental stage requiring a host for a blood meal to progress to the subsequent stage. Tick longevity may extend from months to years, depending on the species and prevailing environmental conditions. Control methods for ticks include chemical acaricides, integrated pest management and biological control strategies, though rising resistance to chemicals emphasises the need for alternative approaches, including the use of natural acaricides (George et al., 2004).

2.2 Rhipicephalus appendiculatus

Rhipicephalus appendiculatus is a well-known hard tick of the Acarinae family (Natsuaki, 2021), also known as the brown eared tick, primarily affecting livestock in Sub-Saharan Africa (Kasaija et al., 2021; Monakale et al., 2024). This parasite flourishes in warm, humid climates where dense vegetation and high host prevalence provide ideal conditions for its survival (Makwarela et al., 2024; Monakale et al., 2024). While it targets various large mammals, it shows a distinct preference for the ears of cattle. It serves as the primary carrier for *Theileria parva*, the pathogen responsible for East Coast Fever, which represents one of the most prevalent diseases in cattle in the region (Young & Leitch, 1981; Makwarela et al., 2025). Transmission dynamics are heavily dictated by tick population density, host availability, and fluctuating environmental factors (Chitombo et al., 2021; (Sungirai et al., 2018).

This species follow a classic three-host life cycle, a known characteristic of ixodid ticks (Higa et al., 2020). The cycle starts by deposition of eggs into the environment, where they hatch into larvae. The hatched larva will then attach to a host, feed, and then drop off to moult into nymphs. The nymphs infest a second host, to obtain a blood meal. They later detach to develop into adults (Showler & Harlien, 2018). Lastly, adult ticks attach to a third host to feed and mate, after which the engorged females drop to the ground to deposit their eggs. Each stage of development is strictly dependent on a blood meal, and the speed of this progression is highly sensitive to external conditions such as temperature, humidity, and the quality of vegetation cover (Sungirai et al., 2023; (Nemaungwe et al., 2023).

Since each stage relies on a different host, *Rhipicephalus appendiculatus* has numerous windows of opportunity to transmit pathogens. However, the larval stage represents a critical vulnerability in the tick's biology (Showler & Harlien, 2018). Unlike adults, larvae possess minimal energy reserves and are exceptionally prone to drying out, relying much on humidity for survival (Nemaungwe et al., 2023). Their high level of exposure in the environment before they find a host makes them an ideal target for intervention.

Focusing control measures on the larval stage makes it possible to effectively curb population growth and disrupt the reproductive cycle. This explains the focus on larval stages in acaricidal studies, including the present research. In Addition, increasing reports of acaricide resistance in *R. appendiculatus* highlight the need for alternative control approaches, particularly those targeting early developmental stages (Chitombo et al., 2021; Evans et al., 2024)

Figure 1 Rhipicephalus appendiculatus



Female Rhipicephalus appendiculatus



Male Rhipicephalus appendiculatus

2.3 *Lipia javanica*

2.3.1 Biology and importance of *Lipia javanica.*



Figure 2 Lipia javanica

Lipia javanica, Burm.f.) Spreng, is a perennial aromatic herb belonging to the *Lamiaceae* family. It is extensively distributed throughout Southern and Eastern Africa, including countries such as Zimbabwe, Malawi, and Madagascar (Maroyi, 2017). It typically grows in grasslands, open woodlands, and disturbed areas, demonstrating strong adaptability to a range of ecological conditions (Maroyi, 2017). The plant is characterised by ovate, highly aromatic leaves and small clusters of white to purplish flowers, which attract pollinators and contribute to local biodiversity.

Lipia javanica is rich in essential oils and some bioactive compounds, including caryophyllene, limonene, other terpenoids, and flavonoids, which are associated with its anti-inflammatory, antimicrobial, and analgesic properties (Mujovo et al., 2008; Maroyi, 2017). Traditionally, its leaves are the most used part of the plant, prepared as infusions, decoctions, or inhalants for the treatment of respiratory infections, fever, and gastrointestinal disorders (Maroyi, 2017). Beyond medicinal applications, *L. javanica* leaves are also used as a culinary herb and herbal tea (Mfengu et al., 2021).

Insecticidal, repellent, and acaricidal activities of *L. javanica* have been demonstrated, largely due to the presence of active components in this plant (Nzira et al., 2009; Madzimure et al., 2011). Previous research by Madzimure et al. (2011) found efficacy against cattle ticks; however, the efficacy was inferior to that of conventional acaricides like Amitraz. Nevertheless, this highlights its suitability as an eco-friendly alternative for pest control in cases when the availability of commercial products is limited.

Different solvents, such as distilled water, methanol, and hexane, have been utilized for the extraction of bioactive components, depending on the polarity of phytochemicals (Mujovo et al., 2008). Organic solvents (e.g. ethanol and methanol) can be considered preferable since they have higher efficacy regarding the extraction of a wide array of phytochemicals, while aqueous extracts reflect more traditional approaches (Maroyi, 2017). Therefore, selection of extraction technique and solvent is a crucial issue, determining the amount and biological activity of the extract.

Despite the fact that *L. javanica* showed promise as a natural remedy with insecticidal activity, there is still an important gap in the investigation of its acaricidal activity against *Rhipicephalus appendiculatus* larvae. Previous studies have indicated moderate activity of *Lipia javanica* (Madzimure et al., 2011), however, comprehensive data on its median lethal concentration (LC_{50}) under controlled experimental conditions, particularly with respect to optimal extraction methods, remain limited.

2.3.2 Phytochemical Composition and Acaricidal Activity

Lipia javanica is documented to contain a range of bioactive compounds, including volatile oils, flavonoids, terpenoids, and phenolic constituents. These phytochemicals underpin its antimicrobial, anti-inflammatory, and pesticidal activities, thereby providing a scientific foundation for its potential acaricidal effects (Madzimure et al., 2011). Notably, flavonoids are recognised for their capacity to inhibit acetyl cholinesterase (AChE)) (Cichon et al., 2024), An enzyme integral to neural function in ticks (Temeyer, 2018). The suppression of AChE activity results in the accumulation of acetylcholine within neural synapses, leading to continuous nerve stimulation, paralysis, and ultimately, mortality. Consequently, flavonoids may exert acaricidal effects primarily through neurotoxic mechanisms following ingestion by a tick. Furthermore, flavonoids are known to induce oxidative stress (Jomova et al., 2025), which can facilitate the generation of reactive oxygen species (ROS) within the tick's body. These ROS molecules can damage key cellular components such as lipids, proteins, and DNA, disrupting essential cellular processes and contributing to tick

death. Additionally, terpenoids such as limonene, pulegone, myrcene, and camphor may influence tick physiology by targeting their nervous system, further supporting their potential role in acaricidal activity.

Terpenoids, including compounds such as limonene, pulegone, myrcene, and camphor, are known to impact ticks by interfering with their nervous system. These substances primarily target GABA (gamma-amino butyric acid) and octopamine receptors, which are critical for muscle coordination and neurotransmission in ticks. The modulation of these receptors—either through blockade or overstimulation—can induce spasms, impair motor control, and ultimately result in tick mortality. Terpenoids also compromise the integrity of the tick's cuticle, the protective outer layer that prevents desiccation (Maroyi, 2017). Disruption of this barrier leads to increased desiccation, culminating in dehydration and death. Due to their rapid action and broad-spectrum toxicity across various developmental stages, terpenoids are considered highly effective acaricidal agents.

Phenolic compounds such as caffeic acid, rosmarinic acid, and chlorogenic acid significantly contribute to the acaricidal properties observed in Lipia javanica. These phenolic paradoxically exert pro-oxidant effects by overwhelming the tick’s antioxidant defence mechanisms, leading to membrane disruption, mitochondrial impairment, and interference with key metabolic enzymes. Furthermore, phenolic constituents may directly induce cytotoxicity by causing structural damage to tick cells and disrupting energy production processes (Madzimure et al., 2011). Additionally, phenolics facilitate the synergistic interaction with flavonoids and terpenoids, enhancing their absorption and amplifying their overall bioactivity.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study Area

This study was carried out under controlled laboratory conditions at the Parasitology Department of the Central Veterinary Laboratories under the Department of Veterinary Technical Services in the Ministry of Lands, Agriculture, Water, Climate and Rural Resettlement in Harare, Zimbabwe. This setting allowed monitoring the experimental conditions such as temperature, humidity and light, which are well known influencers of tick survival and behaviour.

3.2 Source of Research Material

Rhipicephalus appendiculatus larvae were obtained from laboratory-maintained tick colonies at the Central Veterinary Laboratories. The larvae were acclimatised under laboratory conditions for 24 hours prior to experimentation to minimise handling stress. Fresh *Lipia javanica* leaves were harvested from a reliable source free from herbicides and pesticides in Bluffhill Westgate, Harare. The plant material was identified and authenticated by a botanist in the Department of Biological Sciences at University of Zimbabwe using standard taxonomic keys. Staff at the Central Veterinary Laboratories also confirmed that the plant material was *Lipia javanica.*

3.3 Research Design

The research used 2 extraction solvents (70% Ethanol and Distilled water). Each solvent consisted of 3 treatment groups of *Lipia javanica* leaf extract concentrations (30%, 50%, and 100%), a positive control (0.03% Amitraz), and a negative control (distilled water). Each treatment was replicated five times, and approximately 100 *Rhipicephalus appendiculatus* larvae were used per replicate. The treatment packets were suspended in an incubator in order of their percentage concentration, starting with the negative and positive controls.

3.3 Research Procedure.

3.3.1 Preparation of *Lipia javanica* leaf extracts

Collected leaves of *Lipia javanica* were air-dried in a shaded, well-ventilated area until they were completely dry. The dried leaves were subsequently pulverised into a coarse powder using a mortar and pestle. Two 50 g portions of the powder were accurately weighed using a balance scale and macerated separately in air tight reagent bottles containing 500 mL of 70% ethanol and 500ml of distilled water for 48 hours. The mixtures were subsequently filtered using Whatman No. 1 filter paper to remove plant residues. The undiluted filtrates obtained from each made the 100% concentration extracts. Working concentrations of 30% and 50% extract concentrations of *Lipia javanica* were prepared from both the ethanol and distilled water filtrates. This was done by diluting 30ml and 50ml of the respective extracts in 70ml and 50ml of distilled water, respectively.

3.3.2 Preparation of Control Treatments

The positive control solution (0.03% Amitraz) was prepared by dissolving 3 g of powdered Amitraz in 100 mL of distilled water. Distilled water alone served as the negative control.

3.3.3 *Rhipicephalus appendiculatus* larval mortality tests

The acaricidal activity of the respective treatments was evaluated using a modified larval packet test. Whatman No. 1 filter papers were uniformly impregnated with the respective treatment solutions and allowed to dry at 25 °C to ensure complete evaporation of the solvents. Each treatment had 5 filter papers impregnated with it. Dried filter papers were folded into packets with the treated surface facing inward. Approximately 100 *Rhipicephalus appendiculatus* larvae were introduced into each packet using a fine brush. The packets were sealed with clips to prevent larval escape. Control packets were prepared first, and packets with the highest extract concentration were prepared last. The packets were suspended in an incubator in order of their percentage extract concentrations, starting with the controls. Incubation was done at 28 °C for 48 hours. Relative humidity was maintained by placing a container of warm water initially heated to 72°C within the incubator to simulate optimal environmental conditions for larval survival. This was done for *Lipia javanica* leaf extracts prepared using both extraction solvents (70% ethanol and distilled water). Packets treated with 30%, 50%, and 100% concentrations prepared using each solvent were incubated under identical environmental conditions.

3.3.4 Determination of Lethal Concentration 50% (LC_{50})

Lethal concentration 50% (LC_{50}) is the concentration of *Lipia javanica* extract required to kill 50% of *Rhipicephalus appendiculatus* larvae. After incubation, the number of dead larvae in each concentration group was recorded, and average mortality was calculated. The mortality data from the 70% ethanol-obtained *Lipia javanica* extracts were then analysed, using regression analysis, to estimate the concentration corresponding to 50% mortality (LC_{50}) from the dose–response curve. The distilled water-extracted *Lipia javanica* leaf extracts were excluded from LC_{50} estimation.

3.4 Data Collection

After the 48-hour incubation period, packets were opened, and the number of dead larvae was recorded for each packet. Larvae were classified as dead when no movement was observed following gentle mechanical stimulation after the 48 hour incubation period. Larval mortality was determined as the proportion of dead larvae relative to the total number introduced per packet and was expressed as the mean mortality for each treatment group.

3.5 Data Analysis

Statisty software was used for statistical analyses of the data. To assess the effect of extraction solvent on acaricidal activity, a main two-way ANOVA was carried out to evaluate the effects of extraction solvent (70% ethanol and distilled water), extract concentration, and the interaction between extraction solvent and extract

concentration on larval mortality. Separate analyses were performed for ethanol and distilled water extracts to test for significant difference in average larval mortality of *Rhipicephalus appendiculatus* larvae among the treatment groups, (30%, 50%, and 100% *Lipia javanica* extract concentrations) and the positive (Amitraz) and negative (distilled water) controls. Where ANOVA showed significant difference, Bonferroni post hoc comparisons were performed to identify the specific treatment groups that differed significantly. LC_{50} values for the extracts were estimated using dose–response analysis based on larval mortality across the tested concentrations.

CHAPTER 4: RESULTS

4.1 Acaricidal activity of Lipia javanica crude leaf extracts obtained from Ethanol as an extraction solvent against Rhipicephalus appendiculatus larva

The findings showed that larval mortality varied according to extract concentration and extraction solvent, indicating concentration-dependent acaricidal activity of *Lipia javanica*. For the ethanol extracts, mean larval mortality increased with concentration, with the 30%, 50%, and 100% concentrations producing mean mortalities of 13, 23, and 33 dead larvae, respectively. The 30% concentration exhibited the lowest mortality, while the 100% concentration showed the highest mortality among the plant extract treatments, suggesting increased acaricidal effectiveness with increasing concentration. The positive control (Amitraz) produced complete larval mortality with a mean of 100 dead larvae, whereas the negative control (distilled water) showed minimal mortality with a mean of 0.4 dead larvae. Compared with distilled water extracts, ethanol extracts demonstrated greater acaricidal activity, indicating that the extraction solvent influenced the efficacy of *Lipia javanica*. Table 1 presents the mean larval mortality values for the different concentrations and controls.

One-way ANOVA test showed that the larval mortality rate of *Rhipicephalus appendiculatus* differed significantly between the treatments ($F= 1143.64$; $p<0.001$). The Bonferroni test indicated that most of the differences between the 30% *Lipia javanica* treatment and the 50% ($p= 0.011$), 100% ($p= 0.021$), Positive control (Amitraz) ($p<0.001$) and Negative control were statistically significant ($p= 0.004$). The same was true for the 50% concentration treatment when compared to the positive control group ($p<0.001$) and negative control ($p<0.001$). The 100% *Lipia javanica* treatment also showed significant differences when compared to the positive control ($p < 0.001$) and the negative control ($p = 0.001$). There was no significant difference ($p = 0.112$) found between the 50% and 100% treatments. The difference between positive and negative control was the most significant ($p < 0.001$).

Table 1: Mean larval mortalities at the different treatments of *Lipia javanica* extracts obtained using Ethanol as the extraction solvent

Treatment/%	Mean Mortality	Variance	Std. Deviation
30% *Lipia javanica* concentration	13	10	3.16
50% *Lipia javanica* concentration	23	6	2.45
100% *Lipia javanica* concentration	33.2	13.2	3.63
Positive control (Amitraz)	100	0	0
Negative control (distilled water)	0.4	0.8	0.89

4.2 Acaricidal activity of *Lipia javanica* crude leaf extracts obtained from Distilled water as an extraction solvent against *Rhipicephalus appendiculatus* larva

The distilled water extracts of *Lipia javanica* demonstrated a weak, albeit concentration-dependent, acaricidal effect on *Rhipicephalus appendiculatus* larvae. At the highest concentration (100%), the extract yielded a mean mortality of 9.4. The diluted extracts were far less effective; the 50% concentration resulted in a mean mortality of only 2.6, while the 30% concentration similarly exhibited low activity with a mean mortality of 2.2, with some replicates showing no larval deaths at all. The positive control (Amitraz) recorded complete mortality in all pockets, with 100% larval death consistently observed, while the negative control (distilled water) had an average of 0.4 dead larvae.

Analysis of Variance (ANOVA) test confirmed statistically significant effects of treatments on larval mortality of *Rhipicephalus appendiculatus* larva when subjected to *Lipia javanica* aqueous (distilled water) extract concentrations. Effects of treatments were highly significant ($F= 1059.12$, $p < 0.001$), implying large variability in larval mortalities across different treatment concentrations. This implied that almost all variation in larval mortalities could be accounted for by treatment concentration. Bonferroni Post Hoc tests further showed that there were no statistically significant differences in the effects of 30% and 50% extract concentrations (mean difference = -0.4, $p = 1.000$) on larval mortality. Both the 30% and 50% extract concentrations also had no statistically significant effects on larval mortality compared to the negative control ($p = 1.000$). The 100% extract concentration led to more larval mortality compared to other extract concentrations, meaning greater effects of higher concentrations. There were highly significant differences in larval mortalities between all extract concentrations and the positive control (mean difference = 97.8, $p < 0.001$; mean difference = 97.4, $p < 0.001$; mean difference = 90.6, $p < 0.001$). Table 2 below provides information regarding the average larval mortality caused by the different concentrations of *Lipia javanica* extracts, as well as the positive and negative controls used during the process.

Table 2: Mean larval mortalities at the different treatments of *Lipia javanica* obtained using Distilled water as the extraction solvent

Treatment	Mean mortality	Variance	Std. deviation
30% *Lipia javanica* concentration	2.2	3.7	1.92
50% *Lipia javanica* concentration	2.6	6.8	2.61
100% *Lipia javanica* concentration	9.4	12.3	3.51
Positive control (Amitraz)	100	0	0
Negative control (Distilled water)	0.4	0.8	0.89

4.3 Lethal concentration 50 (LC_{50})

The distilled water-extracted *Lipia javanica* leaf extracts were excluded from LC_{50} estimation because they produced insufficient larval mortality across the concentration range tested and did not achieve the minimum mortality level required to establish a reliable dose–response relationship for LC_{50} determination. None of the *Lipia javanica* treatment concentrations tested (30%, 50%, nor 100%), induced 50% or greater mortality in *Rhipicephalus appendiculatus* larvae. Consequently, a formal LC_{50} could not be estimated from these data, as the required response threshold (50% mortality) was not reached at any examined concentration. Amitraz, by contrast, achieved near-complete mortality, while the negative control remained near baseline.

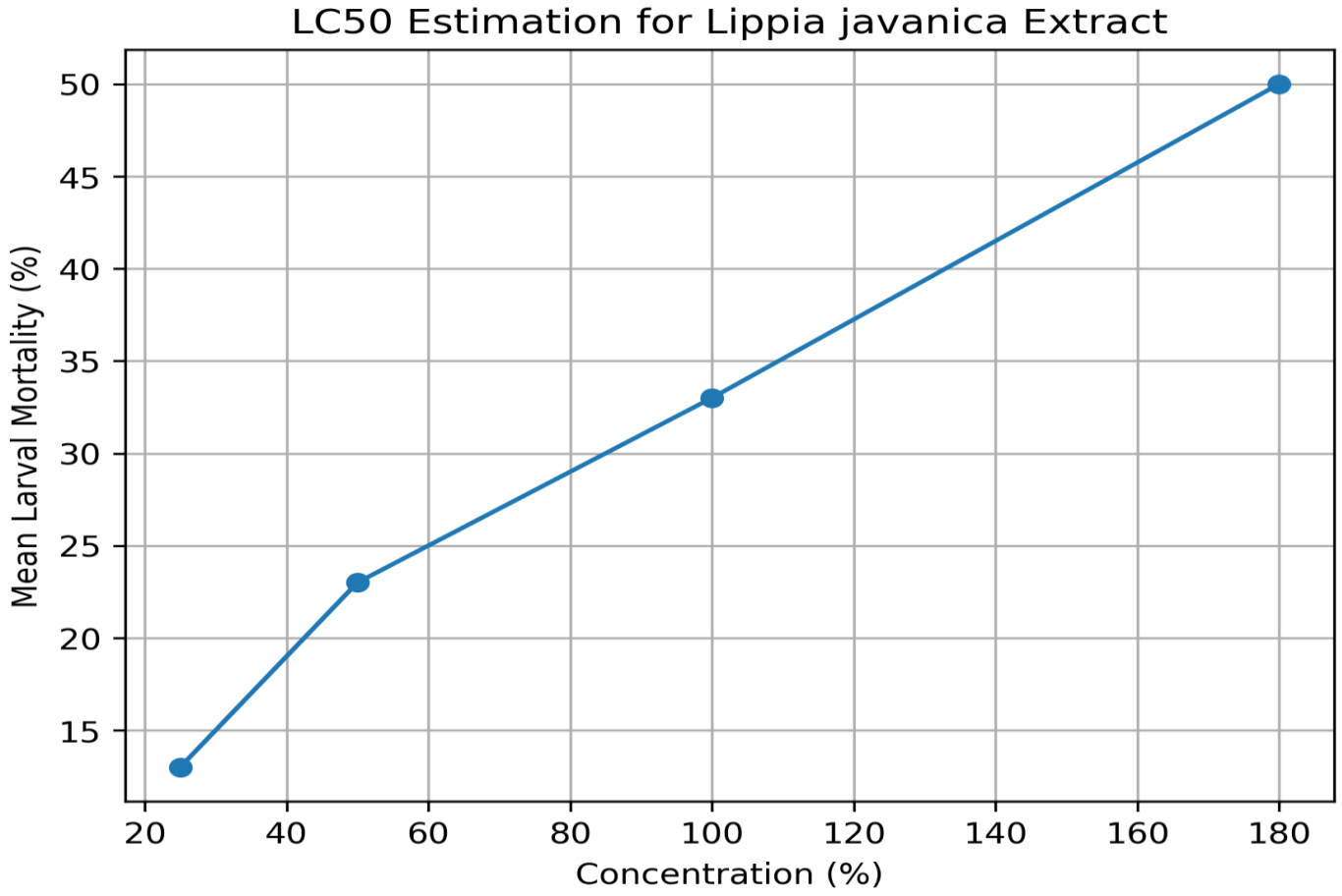

Figure 3: LC50 Estimation for L.javanica leaf extracts

4.4 Effect of Extraction solvent and concentration on Larval Mortality of *Lipia javanica* extracts

The effect of extraction solvent and extracts concentration on larval mortality were analysed using two-way ANOVA. The analysis showed that the main effect of extraction solvent (ethanol versus distilled water) on larval mortality was significant ($F(1, 24) = 290.87$, $p<0.001$), with the ethanol extracts proving to be significantly different from the distilled-water extracts. The concentration of extract also had a significant influence on larval mortality ($F(2, 24) = 55.19$, $p<0.001$): the higher the concentration, the higher the mortality. There was a significant extraction solvent-concentration interaction $F(2, 24) = 13.11$, $p<0.001$, showing that the mortality of larvae varied according to extraction solvent and concentration. The ethanol

extracts of the leaves of *Lipia javanica* were found to be more effective in all concentrations than the extracts from distilled water. Significant differences were found between the mortality of larvae from the ethanol and distilled-water extracts at the same concentrations using Bonferroni post hoc analysis. The 30%, 50% and 100% ethanol extracts were significantly more lethal than their distilled-water counterparts ($p < 0.001$).

CHAPTER 5

5.1 Discussion

This investigation demonstrated that crude leaf extracts of *Lipia javanica* possess acaricidal activity against *Rhipicephalus appendiculatus* larvae, exhibiting a dose-dependent increase in larval mortality with increasing extract concentration. Compared with distilled water extracts, ethanol extracts demonstrated greater acaricidal activity, indicating that the extraction solvent influenced the efficacy of *Lipia javanica.* However, differences in extraction solvent influenced the acaricidal activity observed. The 70% ethanol extracts demonstrated greater activity compared to distilled water extracts, suggesting that extraction efficiency plays an important role in recovering biologically active compounds. The distilled water extracts exhibited weak acaricidal activity across all concentrations tested. Although the both the extracts displayed some concentration-dependent effects, the mortality produced was insufficient to reach 50% mortality within the concentration range tested. The relationship between extract concentration and acaricidal activity observed in the present study confirms known toxicological principles and supports earlier investigations reporting a relationship between acaricidal activity of plants and their phytochemical composition (Madzimure et al., 2011; Maroyi, 2017)). The observed variations among treatment groups ($p < 0.001$) further validate that the extract exerts a biologically significant impact on the survival of *Rhipicephalus appendiculatus* larvae.

Despite its acaricidal properties, *Lipia javanica* was significantly inferior to Amitraz which caused 100% larval mortality. The significant difference between these results underlines the high efficacy of chemical acaricides used in tick control. Unlike plant-based products, these chemicals have been specifically developed for affecting tick neurophysiology.(George et al., 2004). Previous studies also confirmed that although *Lipia javanica* demonstrated some activity, its efficacy was inferior to commercial acaricides, as well (Madzimure et al., 2011). Consequently, although the extract of *Lipia javanica* shows some acaricidal potential, its effectiveness as a standalone control agent appears limited under the conditions tested.

In the ethanol obtained extracts, no difference was noted between larval mortalities in 50% and 100% concentrations of *Lipia javanica* extract. The plateauing in the relationship between extract concentration and acaricidal efficacy might be associated with the saturation point when the increase in concentration leads to neither increased nor decreased biological effect. This observation may relate to limited solubility, instability of the phytochemical mixture or to the fact that the target site has been saturated (Mujovo et al., 2008). Besides, the mixture of phytochemicals in the extract may produce antagonism and reduce their efficacy at higher concentrations.

For the distilled water extracts, the inability to produce substantial mortality may be due to the lower extraction efficiency of water for some bioactive phytochemicals. Water is highly polar (Ben-Amotz & Underwood, 2008) and may have extracted fewer lipophilic compounds such as terpenoids and certain phenolic constituents

that are associated with acaricidal properties (Maroyi, 2017). Since mortality levels remained substantially below 50%, the distilled water extracts were excluded from LC_{50} estimation because meaningful dose–response modelling could not be established within the tested concentration range. The inability of the distilled water extracts to achieve adequate mortality suggests that the solvent may not have efficiently recovered sufficient quantities of active compounds responsible for toxicity against *Rhipicephalus appendiculatus* larvae.

The inability to achieve the level of 50% mortality within the studied range of concentrations, along with LC_{50} being more than 100%, indicates low potency of *Lipia javanica* extract obtained from ethanol as an acaricide. It means that to obtain satisfactory results, either higher concentrations should be considered or the extraction technique needs improvement. Optimization of the extract composition will probably lead to better acaricidal activity of *Lipia javanica.* The current acaricidal activity seems to be related to terpenoids, flavonoids, and phenolic compounds known to be able to affect the physiology of ticks through inhibition of acetyl cholinesterase, modulation of GABA receptors, and creation of oxidative stress. (Temeyer, 2018; Cichon et al., 2024). However, the concentrations of these compounds might have been insufficient for a high-level toxic effect.

The observed outcomes may have because of the crude ethanol used as the solvent for extracting phytochemicals since it dissolves many kinds of compounds. While ethanol is able to extract different phytochemicals effectively, it may not selectively concentrate those that show high acaricidal properties (Mujovo et al., 2008). Moreover, changes in *Lipia javanica* chemical composition due to genetic variation and environmental factors may have affected the biological efficiency of this plant (Maroyi, 2017). Despite being more susceptible than other developmental stages of the ticks, *Rhipicephalus appendiculatus* larvae might contain certain biochemical and physiological defence mechanisms against the phytochemicals of the plant extracts. (Niyonzima et al., 2021).

Based on the results obtained from this study, it is evident that *Lipia javanica* is unable to be a sole agent to exert efficient acaricidal effects. However, it can be a potential complementary agent within the Integrated Pest Management (IPM) approach by virtue of being effective enough, accessible, affordable, and environmentally friendly. By combining plant extracts with lower amounts of synthetic acaricides or any other method of tick control, we would be able to increase effectiveness, delay the development of acaricide resistance, and mitigate environmental effects (Chitombo et al., 2021).

From this experiment, it can be seen that *Lipia javanica* shows dose-dependent acaricidal effects against *Rhipicephalus appendiculatus* larvae, but there is limited potency in crude extract. This finding emphasizes the importance of conducting further studies to improve extraction procedures, identify active compounds,

and test synergistic formulations. These improvements could increase the acaricidal potential of *Lipia javanica* and facilitate its implementation in tick control programs as an environmentally friendly option.

5.2 Conclusions

The study demonstrated that *Lipia javanica* leaf extract has dose-dependent acaricidal properties against *Rhipicephalus appendiculatus* larvae. Despite the high concentration producing high mortality rates, the *Lipia javanica* extract did not reach a 50% mortality rate at the concentration range used in this study. The calculated LC_{50} (≈182.35%) exceeded the maximum concentration used, which suggests limited efficacy of the extract under the experimental conditions. In contrast, the positive control (Amitraz) showed total effectiveness in eliminating *Rhipicephalus appendiculatus* larvae compared to *Lipia javanica* extract. Thus, synthetic acaricides show superior efficacy compared to natural substances. Future studies should investigate optimization of extraction methods and other parts of *Lipia javanica* (e.g. roots), including exploring combinations with other natural extracts to enhance efficacy. Further studies involving higher concentrations, refined extraction methods, and advanced toxicological analyses (e.g., probit analysis) are recommended to better characterise its dose–response relationship and practical applicability.

5.3 Recommendations

Further studies should investigate higher concentrations of *Lipia javanica* extract to determine a more accurate LC_{50} within an observable range. Improved extraction methods (e.g., solvent extraction, fractionation) should be explored to enhance the concentration of active phytochemicals. Advanced statistical approaches such as probit analysis should be applied for a more precise determination of dose–response relationships. The potential synergistic effects of combining *Lipia javanica* with other plant extracts or reduced doses of synthetic acaricides should be evaluated. Further research should assess the field applicability, safety, and environmental impact of the extract under natural conditions. Investigation into the active compounds responsible for acaricidal activity is recommended to support the possible formulation of more effective bio-based acaricides.

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APPENDICES

Appendix 1 number of dead larva per *Lipia javanica* concentration from Ethanol

Pocket number	30%	50%	100%	Positive control(amitraz)	Negative control
1	9	20	36	100	0
2	13	24	30	100	0
3	11	24	38	100	0
4	15	21	32	100	0
5	17	26	30	100	2

Appendix 2 Number of dead larva per % *Lipia javanica* concentration from distilled water

Pocket number	30%	50%	100%
1	3	0	10
2	5	6	9
3	0	3	15
4	2	0	7
5	1	4	6

Appendix 3 The Bonferroni post-hoc test summary table for ethanol extracts

		Mean diff.	Std. Error	95% CI upper limit	P	95% CI lower limit	
30%	50%	-10	1.183	-8.452	.011	-13.29	-6.71
30%	100%	-20.2	2.871	-7.037	.021	-28.17	-12.23
30%	positive	-87	1.414	-61.518	<.001	-90.93	-83.07
30%	negative	12.6	1.166	10.804	.004	9.36	15.84
50%	100%	-10.2	2.289	-4.456	.112	-16.56	-3.84
50%	positive	-77	1.095	-70.291	<.001	-80.04	-73.96
50%	negative	22.6	0.872	25.924	<.001	20.18	25.02
100%	positive	-66.8	1.625	-41.113	<.001	-71.31	-62.29
100%	negative	32.8	1.855	17.685	.001	27.65	37.95
positive	negative	99.6	0.4	249	<.001	98.49	100.71

Appendix 4 One-way ANOVA table for ethanol extracts

	Type III Sum of Squares	df	Mean Squares	F	p	η^2
Treatment	30237.84	4	7559.46	1143.64	<.001	1
Residual	105.76	16	6.61			

Appendix 5 Bonferroni post hoc table

Comparison	**Mean Difference**	**95% CI Lower**	**95% CI Upper**	**p-value**	**Interpretation**
30% Ethanol vs 30% Distilled water	**10.8**	**5.84**	**15.76**	**<0.001**	**Significant**
50% Ethanol vs 50% Distilled water	**20.4**	**15.44**	**25.36**	**<0.001**	**Significant**
100% Ethanol vs 100% Distilled water	**23.8**	**18.84**	**28.76**	**<0.001**	**Significant**
30% Ethanol vs 50% Ethanol	**10.0**	**5.04**	**14.96**	**<0.001**	**Significant**
50% Ethanol vs 100% Ethanol	**10.2**	**5.24**	**15.16**	**<0.001**	**Significant**
30% Ethanol vs 100% Ethanol	**20.2**	**15.24**	**25.16**	**<0.001**	**Significant**
30% Distilled water vs 50% Distilled water	**0.4**	**-4.56**	**5.36**	**>0.05**	**Not significant**
50% Distilled water vs 100% Distilled water	**6.8**	**1.84**	**11.76**	**<0.05**	**Significant**
30% Distilled water vs 100% Distilled water	**7.2**	**2.24**	**12.16**	**<0.05**	**Significant**

Appendix 6 Two way ANOVA Table

Source of variation	Sum of Squares (SS)	df	Mean Square (MS)	F-value
Extraction solvent	2520.83	1	2520.83	290.87
Concentration	956.60	2	478.30	55.19
Solvent × Concentration	227.27	2	113.63	13.11
Error (Residual)	208.00	24	8.67	
Total	3912.70	29		

Appendix 7 One way ANOVA table for distilled water extracts

Source of Variation	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Between Groups	30556.16	4	7639.04	1059.12	<0.001
Within Groups (Error)	144.30	20	7.22		
Total	30700.46	24			

Appendix 8 Post Hoc table for Distilled water extracts

Comparison	Mean Difference	95% CI Lower	95% CI Upper	p-value	Interpretation
30% Distilled water vs 50% Distilled water	0.4	-4.08	4.88	>0.05	Not significant
30% Distilled water vs 100% Distilled water	7.2	2.72	11.68	<0.01	Significant
50% Distilled water vs 100% Distilled water	6.8	2.32	11.28	<0.01	Significant
30% Distilled water vs Positive control	97.8	93.32	102.28	<0.001	Significant
50% Distilled water vs Positive control	97.4	92.92	101.88	<0.001	Significant

Comparison	Mean Difference	95% CI Lower	95% CI Upper	p-value	Interpretation
100% Distilled water vs Positive control	90.6	86.12	95.08	<0.001	Significant
30% Distilled water vs Negative control	1.8	-2.68	6.28	>0.05	Not significant
50% Distilled water vs Negative control	2.2	-2.28	6.68	>0.05	Not significant
100% Distilled water vs Negative control	9.0	4.52	13.48	<0.001	Significant
Positive control vs Negative control	99.6	95.12	104.08	<0.001	Significant

Appendix 9 Pictures showing some stages of plant extracts preparation

